Exploring the diversity and antimicrobial potential of predatory bacteria from Indonesian mangroves

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Von Senlie Octaviana
Aus Salatiga / Indonesien

1. Referent: Professor Dr. Michael Steinert
2. Referent: Privatdozent. Dr. Joachim Wink
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Abbreviations

BLAST	The Basic Local Alignment Search Tool
DSMZ	German Collection of Microorganisms and Cell Cultures GmbH
GGDC	Genome to Genome Distance Calculator
GTR+GAMMA	General Time Reversible, gamma distributed rate variation among sites
HPLC	High Performing Liquid Chromatographie
HPLC-UV-HRESIMS	High Resolution Mass Spectra
MEGA X	Molecular Evolutionary Genetics Analysis
NCBI	National Center for Biotechnology Information
PAUP*	Phylogenetic Analysis Using Parsimony
PCR	Polymerase Chain Reaction
RaxML	Randomized Accelerated Maximum Likelihood
RDP	Ribosomal Database Project
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SEM	The standard error of the mean
TNT	Tree analysis using New Technology
XAD-resin	a hydrophobic cross-linked polystyrene copolymer resin, widely used to adsorb soluble organic compounds from organic solvent

ABSTRACT

Mangroves are highly complex coastal ecosystems, which harbor manifold microbial communities including diverse bacterial taxa. The interactions between predatory bacteria such as myxobacteria and mangroves are hitherto poorly understood. Using a polyphasic approach to characterize predatory bacteria from Indonesian mangroves based on culture-dependent and culture-independent approaches and subsequently evaluation of their potential for antimicrobial activity are the aims of our study. We obtained the data on identified cultivable predatory bacteria, 25 isolates of myxobacteria and two other isolates of gliding bacteria which belong to the order *Cytophagales*, using partial 16S rRNA gene sequences. These 25 myxobacteria isolates were affiliated to six genera: *Myxococcus*, *Corallococcus*, *Archangium*, *Chondromyces*, *Racemicystis*, and *Nannocystis* of the order *Myxococcales*. However, the culture-independent approach showed that myxobacteria communities are more diverse than assumed. The polyphasic approach led to the identification of a new strain, *Ohtaekwangia* 313MSO from Indonesian mangrove at Jakarta. The evaluation of six housekeeping genes for multilocus sequences analysis (MLSA) of *Myxococcus* spp. isolates revealed genetically distinct six *Myxococcus* strains. Thirteen crude extracts showed moderate activities against at least one human pathogenic microorganism and the crude extract of *Racemicystis persica* strain 503MSO indicated a novel compound, which has not been reported in the myxobase database yet. Therefore, identification of this compound is needed for further study.

Keywords: Antimicrobial, Indonesian, Mangroves, MLSA, Polyphasic, Predatory Bacteria

ABSTRAKT

Mangroven sind hochkomplexe küstennahe Ökosysteme, die vielfältige mikrobielle Gemeinschaften, einschließlich verschiedener bakterieller Gruppen, beherbergen. Die Wechselwirkungen zwischen räuberischen Bakterien wie Myxobakterien und Mangroven sind bisher kaum bekannt. Die Durchführung eines mehrphasigen Ansatzes zur Charakterisierung räuberischer Bakterien aus indonesischen Mangroven auf der Grundlage kulturabhängiger und kulturunabhängiger Ansätze und die anschließende Bewertung ihres Potenzials hinsichtlich der antimikrobiellen Aktivität sind die Ziele dieser Studie. Wir präsentieren Daten zu identifizierten kultivierbaren räuberischen Bakterien von 25 Isolaten der Myxobakterien und zwei weiteren Isolaten von gleitenden Bakterien, die zur Ordnung *Cytophagales* gehören, unter Verwendung partieller 16S-rRNA-Gensequenzen. Diese 25 Myxobakterien-Isolate wurden sechs Gattungen zugeordnet: *Myxococcus*, *Corallococcus*, *Archangium*, *Chondromyces*, *Racemicystis* und *Nannocystis* aus der Ordnung der *Myxococcales*. Ein kulturunabhängiger Ansatz zeigte jedoch, dass Myxobakteriengemeinschaften vielfältiger sind als angenommen. Der mehrphasige Ansatz führte zur Isolierung eines neuen Stammes, des Stammes *Ohtaekwangia* 313MSO, aus Jakarta, genauer aus den indonesischen Mangroven. Die Bewertung von sechs Housekeeping-Genen für die multilocus-Sequenzanalyse (MLSA) von *Myxococcus* spp. zeigten sechs genetisch unterschiedliche *Myxococcus*-Stämme. Dreizehn Rohextrakte zeigten mäßige Aktivitäten gegen mindestens einen der humanpathogenen Mikroorganismen, und der Rohextrakt aus *Racemicystis persica* Stamm 503MSO enthält eine neue Verbindung, über die in der Myxobase Datenbank noch nicht berichtet wurde. Daher sind zur Identifizierung dieser Verbindung weitere Untersuchungen erforderlich.

Schlüsselwörter: Antimikrobiell, indonesische Mangroven, MLSA, mehrphasig, räuberische Bakterien

CHAPTER 1. INTRODUCTION

1.1. New Diseases: Challenges and Solutions

Since the modern antibiotic era began in the early 20th century, it has positively changed the course of medicine and has saved millions of people's lifes worldwide. However, new resistance to antimicrobial drugs has emerged rapidly. New antibiotic resistance mechanisms, which cause new infection diseases, have been resulting in prolonged illness, disability and deaths [1].

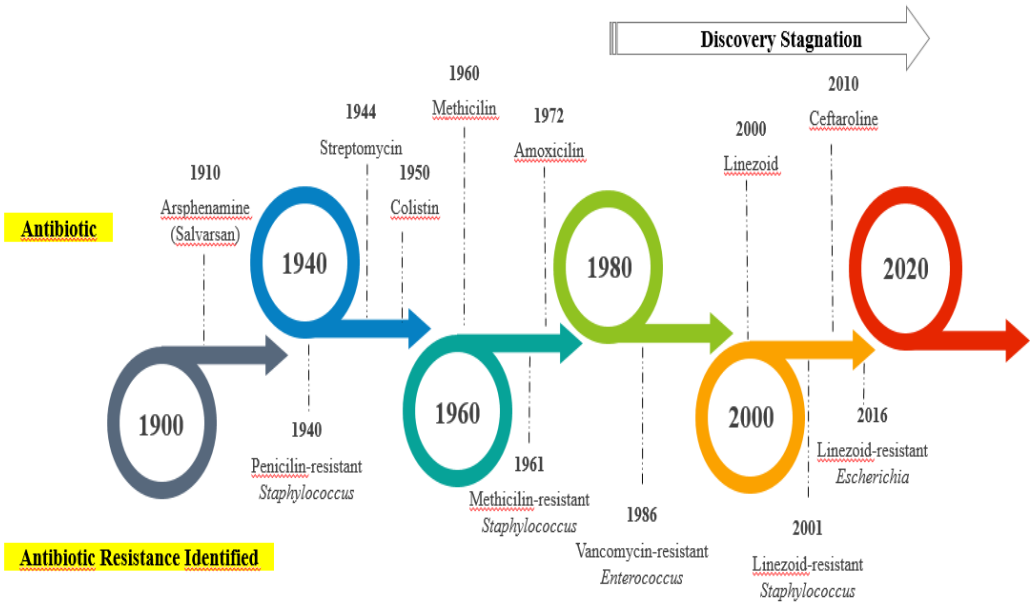


Figure 1. Timeline of antibiotic discovery (upper) and of evaluation of drug-resistant bacteria (below). Adapted from Rončević et al [1].

Since sensitivity of *Staphylococcus* to penicillin in 1940, methicillin has been used from 1960 to exhibit potent activity against *Staphylococcus*. However, antibiotic resistance emerged rapidly only two years after its application (Figure 1), whereas resistance of *Enterococcus* to vancomycin was observed by 1986 (Figure 1). Infectious diseases could not be easily controlled and finding of promising compounds had slowed down in the 2000s. Resistance of *Staphylococcus* to linezoid emerged shortly after one year being applied in 2000 (Figure 1). In recent years, due to the rapid increase in resistance of *Enterobacteriaceae* (Gram-negative bacteria) to colistin, a beta-lactam antibiotic, serious concern has occurred in

clinical setting (Figure 1). In the early 2020, infectious coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has also spread rapidly throughout more than 190 countries. The next decade is likely to witness an endangering rise in antimicrobial resistances.

Current solutions to antimicrobial resistance are challenging. However, new secondary metabolites from natural products have a big potential to offer treatment for infectious diseases or even antimicrobial resistance. Natural products are produced by a variety of organisms, such as plants, animals and microorganisms. Microbial natural products (MNP) have become promising candidates as a source of bioactive compounds since Alexander Fleming in 1928 observed a rare *Penicillium notatum* strain secreting a "mould juice", named "penicillin", which destroyed staphylococcal bacteria. Later, Howard Florey, Ernest Chain and their colleagues from Oxford University developed penicillin into a lifesaving drug.

Natural products have been a prolific source for numerous medical agents with various biological activities including antimicrobial activities and therapeutic applications for human diseases [2]. Although the available number of natural products is low compared to synthetic medical agents, 69 % of all antibacterial agents have been reported to be derived from natural products [2].

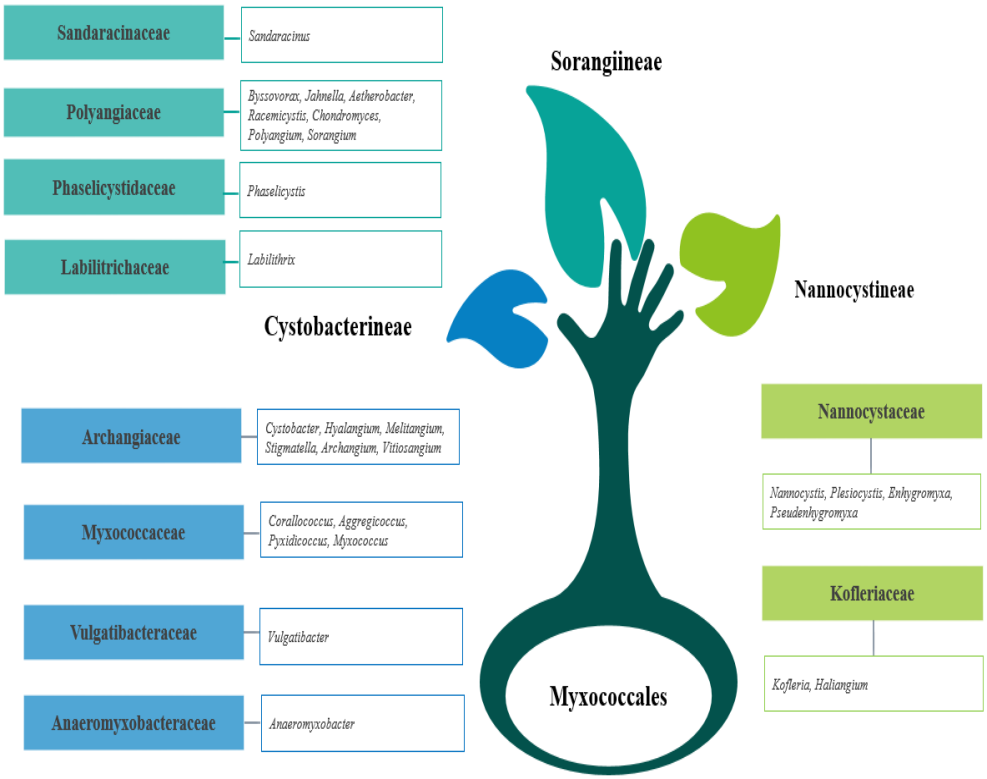
Research on natural products is based on top-down and bottom-up approaches [2]. In top-down research, natural products are found after isolation of microorganisms from diverse environments and they are characterized to be active against pathogenic microorganisms. Bottom-up research starts from the manipulation of microorganisms along with the biotechnology methods such as genome mining, mutation, microbial transformation technique to produce targeted novel compounds and derivatives [2]. In the present study, top-down research has been chosen in order to isolate microbial natural products from Indonesian mangroves ecosystems.

1.2. Microbial Natural Products

1.2.1. Myxobacteria

Over the last decades, myxobacteria are one of the fascinating microbes with an extraordinary life style [3]. They live predominantly in the soil, move by gliding and feed cooperatively in predatory groups or use cellulose. When nutrients are deficient, myxobacterial cells start to form species-specific fruiting bodies. Within these fruiting bodies, vegetative cells convert to dry-resistant myxospores and could survive for decades. If the conditions become better, spores germ out and the life cycle starts over again [4].

Worldwide myxobacteria are distributed in diverse habitats such as in soils, decaying plant materials, and dung of herbivores even under extreme conditions [5, 6]. The order *Myxococcales* has been divided phylogenetically into three suborders: *Cystobacterineae*, *Sorangiineae* and *Nannocystineae,* which comprises 10 families, 29 genera and 65 species (Figure 2).



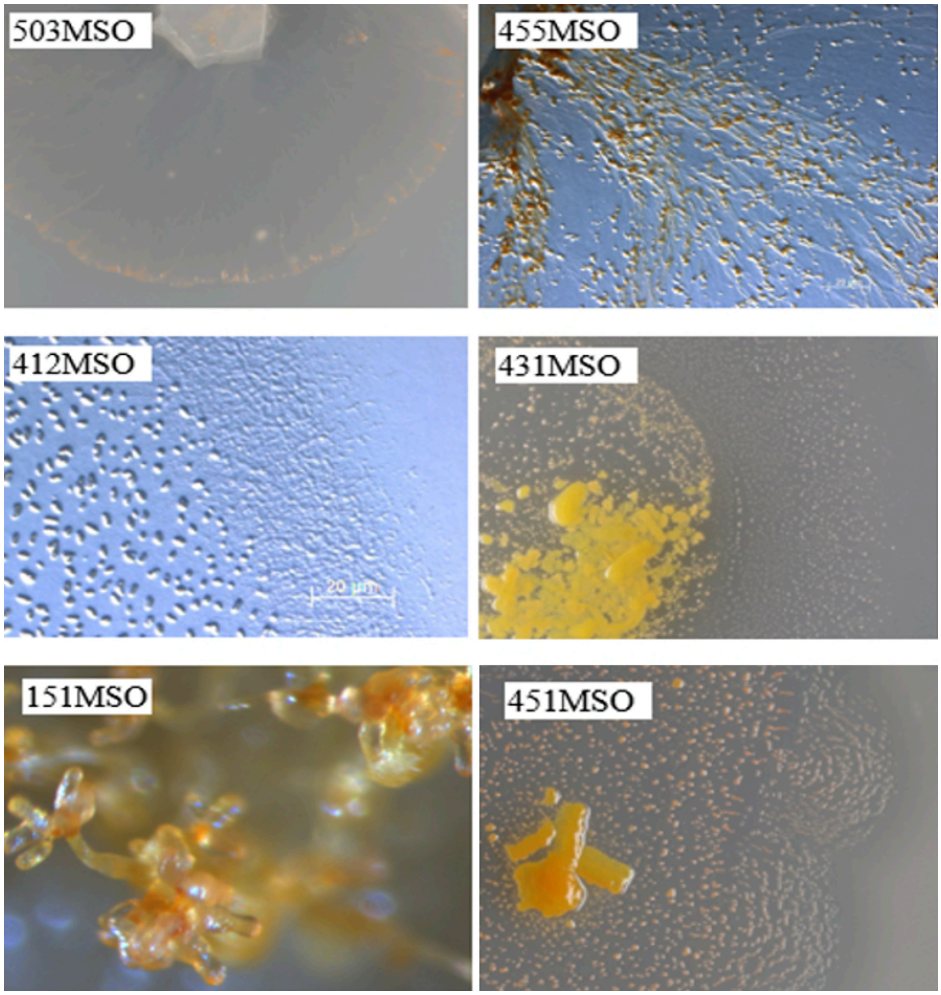


Figure 2. Monophyletic order *Myxococcales (Delta-Proteobacteria*), adapted from Mohr [6] and Some myxobacteria isolates from Indonesia Mangrove were obtained in this study. Swarming area of strain 503MSO and fruiting bodies of strains 455MSO, 412MSO, 413MSO, 451MSO on VY/2 agar medium. Strain 151MSO on Stan21 agar medium.

Approximately 600 derivatives and 100 new carbon skeleton metabolites from myxobacteria have been isolated and identified so far. Ambrucitin is the first antifungal isolated from strain *Sorangium* sp. [6]. At the beginning of 1990s, ephotilones isolated from *Sorangium* sp. showed anti-neoplastic activity [7]. Furthermore, they were tested in clinical trials as anticancer chemotherapy agents [8, 9]. An array of compounds isolated from myxobacteria has been proven to exhibit diverse biological activities such as antibacterial, antifungal, antimalarial, anti-oxidative and antiviral activities [10–13]. Table 1 summarize antimicrobial compounds from Myxobacteria with major biological effects. The chances for isolation of novel antimicrobial compounds from new myxobacteria genera are

bigger than those within the known genera [14]. Therefore, the search for novel myxobacteria is still an extremely promising approach for detection of new antibiotics.

Table 1. Antimicrobial compounds isolated from Myxobacteria. Adapted from Till et al [15].

Major Biological Effect	Myxobacterial strains	Compound
Bacterial RNA polymerase	*Corallococcus* sp.	Corallopyronin
	Myxococcus sp.	Myxopironin
	Sorangium sp.	Ripostatin A-C; Sorangicins; Etnangien
Bacterial protein biosynthesis	*Cystobacter* sp.	Althiomycin
	Myxococcus sp.	Althiomycin; Myxovalargins
	Angiococcus sp.	Angiolam A
Targeting the respiratory chain	*Stigmatella* sp.	Aurachins; Stigmatellin; Myxalamid
	Sorangium sp.	Thuggacins
	Chondromyces sp.	Crocacin
	Myxococcus sp.	Myxalamid; Myxothiazol
Influencing biofilm formation	*Sorangium* sp.	Carolactons
Targeting the type II signal peptidase LspA	*Myxococcus* sp.	Myxovirescins
Anti-fungal	*Chondromyces* sp.	Ajudazol; Crocacin
	Stigmatella sp.	Aurafuron; Myxalamid; Stigmatellin
	Archangium sp.	Aurafuron
	Byssovorax sp.	Cruentaren
	Hyalangium sp.	Hyaladione
	Myxococcus sp.	Myxalamid; Myxothiazol; Myxochromide
Cytotoxic	*Chondromyces* sp.	Apicularen
	Archangium sp.	Aurafuron
	Stigmatella sp.	Aurafuron; Stigmatellin
	Byssovorax sp.	Cruentaren
	Hyalangium sp.	Hyaladione
	Myxococcus sp.	Myxothiazol; Myxochromide

	Chondromyces sp.	Saframicin Mx1
	Sorangium sp.	Tartrrolon B
Inhibiting vascular type ATPase	*Chondromyces* sp.	Apicularen
Immunosuppressive	*Archangium* sp.	Argyrin
Anti-viral	*Myxococcus* sp.	Myxochelin
Unknown mode of action	*Chondromyces* sp.	Chondrochlorens
	Sandaracinus sp.	Indiacens A and B
		Maracin A; Maracen A;
	Sorangium sp.	Sorangiadenosine; Sulfangolids; Kulkenon
	Nannocystis sp.	Nannochelins
	Cystobacter sp.	Roimatacene

1.2.2. Other gliding bacteria

Recent developments in new drug discovery regarding myxobacteria have led to the isolation of diverse bioactive metabolites. Gliding bacteria as a predatory group have gathered more attention in natural products research. In the history of predatory bacteria, Reichenbach and his team have isolated a number of predatory bacteria, which all belong to Gram-negative bacteria and are uniquely motile by gliding on surfaces [16, 17]. They are grouped into the class of *Flexibacteriae* with two orders *Cytophagales* and *Myxobacterales*, which are later classified to the Phylum *Bacteroidetes* [17].

During the golden era of antibiotics, members of *Cytophagales* showed promising results for anti-infective. The genus *Flexibacter* for example, has been reported to produce formacidins which are biologically active against some species of *Pseudomonas*, *Proteus* and *Alcaligenes* [18] and topostins are mammalian DNA topoisomerase I inhibitors [19]. Another gliding bacterium *Cytophaga* sp., which produced katanosin peptides and macrolides has been proven to exhibit inhibition against MRSA, whereas *Flavobacterium* sp. produced the beta lactam antibiotic deacetoxycephalosporin C [20]. In 2008, *Lysobacter capsici* has been revealed to exhibit strong antimicrobial activity against *Colletotrichum gloeosporioides*, *Colletotrichum coccodes*, *Colletotrichum orbiculare* and *Pythium ultimum* [21]. Moreover, gliding bacteria from the strain collection of the Helmholtz Center for

Infection Research (HZI) have been reported to show promising results for discovery of anti-infectives. Okanya *et al*. [22] isolated marinoquinoline A and five derivatives B-F from PWu25 strain, which is closely related to the strain *Ohtaekwangia kribbensis.* These compounds have activity against microbial pathogens, tropical parasites such as *Plasmodium falciparum* and show moderate cytotoxicity to growing mammalian L6 muscle cell lines.

1.3. Polyphasic Approach Leads to a Robust Classification System

The classification of microorganisms based on a combination of genotypic, chemotaxonomic and phenotypic data is known as a polyphasic approach. Since 1970 the polyphasic approach has been used by microbiologists for characterization of novel species and it provides better taxonomic resolution for microbial systematics (Figure 3).

The 16S rRNA gene is commonly used for phylogenetic studies as it is highly conserved with variable and hypervariable regions and 16S rRNA sequences from all bacteria have similar functionalities and sequences sizes which are relatively easy to sequence [23]-[24].

Whole genome sequencing of isolated bacterial strains reveals more information than the 16S rRNA gene sequences. It delivers not only information about the percentage of guanosine and cytosine (GC-content) but also individual genes of interest (such as the antibiotic-resistant genes), single nucleotide polymorphism (SNPs) and highly related lineages of bacteria [25]. However, whole genome sequences of many prokaryotes have a lack of information in public databases. Furthermore, in many cases the data of whole genome sequencing of some species did not provide type strains whereas in prokaryotic taxonomy type strains have an important information in order to define novel species [26].

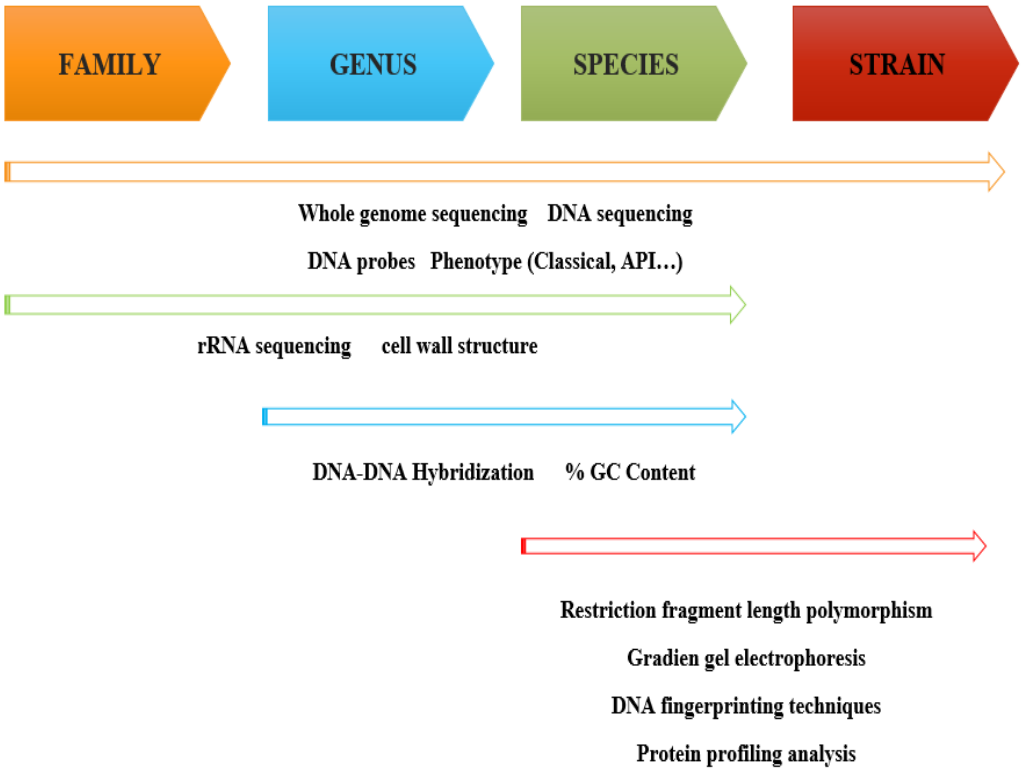


Figure 3. Diagrammatic representation of techniques in polyphasic approach. Adapted from Das et al. [24]

Compared to whole genome sequencing, multilocus sequences typing (MLST) or multilocus sequences analysis (MLSA) provides an alternative method to establish taxonomic position among closely related strains and species [27]. These tools measure DNA sequences variations using a set of housekeeping genes to characterize a strain. In MLST, the specific combination of alleles for each strain and sequences type designations are used whereas the number of nucleotide profiles differences between alleles is ignored. Meanwhile, MLSA is obtained to elucidate the phylogenetic relationships of species within genera [24][27].

In polyphasic taxonomy, chemotaxonomic methods are also used to analyze cell wall and membrane constituents by different chromatographic techniques up to genus level [24]. The most common chemotaxonomic technique is concerned to the distribution of specific chemical of constituents the cell walls such fatty acids, isoprenoid quinones, and polyamines. Phenotypic methods are also used to characterize organisms in different substrates and under different conditions [28].

Hence, the goal of classification of microorganisms is to develop a system for identification of various bacterial species and of hierarchical grouping of species on the basis of their correlated characters [23]. In this study, a polyphasic strategy has been used to describe new bacterial species.

1.4. Metagenomics Analysis of Myxobacterial Communities

The ecological distributions of myxobacterial communities from the classical isolation and molecular survey (independent-cultivation) have been reviewed frequently [4–6, 29–35]. A preliminary study favored the use of specific primers and probes to determine the myxobacterial diversity from soil samples [31].

Since 2011, it has been a rapid rise in the use of some metagenomics methods such as PCR-DGGE (Denaturing Gradient Gel Electrophoresis), quantitative RT (Real Time) –PCR and clone bank analyses [32, 34, 36, 37]. Metagenomics is a microbial communities analyzing method based on genetic analysis of whole microbial genome from environments [38]. Metagenomics can deliver information which microorganisms are present in a sample and their relative abundance. It also provides insight into genomic linkages between community function and structure [38].

Nowadays, next generation sequencing (NGS) method of hypervariable regions of the 16S rRNA gene is a tool to reveal more profound insight into the diversity of microbial communities. Linares-Otoya *et al*. [39] used NGS to report predatory bacteria from the Peruvian Coastline and mentioned that the microbiome present in this region is a promising source for heterotrophic bacterial strains having potential for bioprospecting of antibiotics.

Overall, independent-cultivation revealed an enormous diversity of hitherto uncultivated bacteria cluster. However, for production of secondary metabolites and large-scale fermentation, the producer strains have to be isolated from natural sources [6].

1.5. Mangrove Microbial Richness

Mangroves are small forest trees in brackish water at transitional area between coastal and terrestrial zones [40]. These unique habitats overcome a wide range of salinity, oxygen level change and nutrient. The impact of unique environments provides a habitat harboring diverse groups of microorganisms. The common bacterial groups present in mangroves are among others: N2-fixing bacteria, photosynthetic oxygenic, methanogenic, decomposers bacteria as well as *actinobacteria*. Not only bacteria, but also various groups of fungi and algae are present in mangrove habitats. The structure of microbial communities in mangroves represents the first step towards a better understanding of their existence in ecosystem functions [40].

Mangroves have been proven to be an important ecosystem of industrial applications such as enzymes, paper manufacturing, phycocolloids like agar, carrageenan and alginate, agricultural production, bio surfactants, natural products and other commercially important products as well as the production of various secondary metabolites [41–43]. Antimicrobial compounds isolated from saprophytic fungi, which build a mutualistic relationship with the mangrove trees such as *Aigialus parvus* strain BCC5311 have been reported to produce aigialomycins A-E [44]. Likewise, enalins A and B, which are isolated from *Verruculina enalia,* have been shown to exhibit antimicrobial, antifungal, phytotoxic and antidiabetic activities. In the groups of bacteria, actinobacteria such as *Streptomyces* spp. isolated from Surabaya, Indonesia were found to produce antimicrobial compounds against both of Gram-positive and -negative bacteria [45]. Iizuka *et al*., [46] mentioned that four genera of myxobacteria from marine habitats have been isolated. Two of them, *Haliangium* and *Enhygromyxa,* produced haliangicin, salimabromide, salimyxins, enhygrolides, and haliamide [46]. It has been assumed that saline environments like coastal harbors have an enormous potential for the isolation of new antibiotics from myxobacteria [6].

Figure 4. Sampling areas in this study. Left: Taman Muara Angke Mangrove, Jakarta (MA) 6º105321'N 106º735578'E ; Center: Muara Tawar Mangrove, Bekasi (MT) 6º088772'N 107º735578'E; Right: Mangrove Api-Api, Yogyakarta (MK) 7º894662'S 110º02554'E. These maps adapted from www.d-maps.com.

Mangroves have been neglected for their microbial richness, Jiang *et al*., [47] mentioned that the NGS method has succeeded to prove bacteria with high abundancy in mangroves. Liu *et al* [35] also mentioned an impressive insight to myxobacterial communities over the world. Due to the rare information about myxobacterial communities from Indonesian mangroves, in the present study, myxobacterial communities have been evaluated using specific primers for myxobacteria.

1.6. Outline of work

Indonesia is the home of the world's largest mangrove area, which covered 20.9 % of the world global mangrove area total. Moreover, Indonesia is one of the world's biodiversity hotspots, has 95.000 km of coastlines and over 17.500 islands [40, 48,

49]. This biodiversity certainly harbors a great potential for new secondary metabolites produced by myxobacteria.

The object of our study was to isolate predatory bacteria, especially myxobacteria from Indonesian mangroves. Their diversity and potential for antimicrobial activity have been studied. Furthermore, a polyphasic approach has been used to characterize the isolates.

Totally, the author has four projects in this study. Firstly, myxobacterial diversity from MA, MT and MK sampling sites (Figure 4) has been evaluated by Illumina sequencing with specific primers for myxobacteria. In the second project, myxobacteria from Indonesian mangroves have been isolated and identified by 16S rRNA gene sequencing. All strains have been tested for antimicrobial activity. In the third project, 66 different type strains of myxobacteria from the microbial culture collection at HZI and myxobacterial isolates from mangroves have been analyzed with multi locus sequences analysis (MLSA) for phylogeny of the *Myxococcales*. In the last project, we focused on the designed 313MSO strain, a new species of gliding bacteria. Therefore, the designed 313MSO strain has been compared to type strains *Ohtaekwangia koreensis* 3B2 DSM25262^T and *Ohtaekwangia kribbensis* 10AO DSM25221^T by polyphasic approach and tested for antimicrobial activity.

1.7. Aim of the study

The aims of our study are defined as follows:

1. To gather information about myxobacterial diversity from three different areas.
2. To obtain myxobacterial strains from Indonesian mangroves and evaluate their potential for antimicrobial activity.
3. To obtain a higher expansion of the phylogenetic tree of *Myxococcales* strains.

4. To obtain characteristic of the new strain 313MSO, whose DNA sequence is closely related to the DNA sequence of two type strains of the genus *Ohtaekwangia* and evaluate its potential for antimicrobial activity.

1.8. Sample collection

Totally 34 probes from three different locations of Indonesian mangroves were collected in 2017/2018 (Figure 4, Table 2). Approximately 250 g of each sample were collected from the upper sediment and filled in sterile zip lock plastic bags by using a sterile spatula. All the samples were preserved on dry ice immediately after sampling and transported to the laboratory at the Research Center for Chemistry LIPI, Serpong, Indonesia. To reduce contamination, they were dehydrated by open air at room temperature and then transported to Microbial Culture Collection at the HZI, Braunschweig, Germany.

Table 2**.** Various sites selected from sample collection.

*JW = Joachim Wink; KIM = Kathrin I Mohr; SO = Senlie Octaviana

Samples	Geographic of Origin	Host of Organism	Location	Taken	Collector*
M11	Muara Angke, North Jakarta	Soil	Conservation	2017	JW, KIM
M12	Muara Angke, North Jakarta	Soil	Conservation	2017	JW, KIM
M13	Muara Angke, North Jakarta	Wood flakes	Conservation	2017	JW, KIM
M14	Muara Angke, North Jakarta	Leaf flakes	Conservation	2017	JW, KIM
M15	Muara Angke, North Jakarta	Soil	Conservation	2017	JW, KIM
M16	Muara Angke, North Jakarta	Leaf flakes	Conservation	2017	JW, KIM
M17	Muara Angke, North Jakarta	Wood flakes	Conservation	2017	JW, KIM
M18	Muara Angke, North Jakarta	Soil	Conservation	2017	JW, KIM
M19	Muara Angke, North Jakarta	Soil	Conservation	2017	JW, KIM
M20	Muara Angke, North Jakarta	Wood flakes	Conservation	2017	JW, KIM
M21	Muara Angke, North Jakarta	Soil	Conservation	2017	JW, KIM

M22	Muara Angke, North Jakarta	Wood flakes	Conservation	2017	JW, KIM
M23	Muara Angke, North Jakarta	Soil	Conservation	2017	JW, KIM
M24	Muara Angke, North Jakarta	Soil	Conservation	2017	JW, KIM
M25	Muara Angke, North Jakarta	Leaf flakes	Conservation	2017	JW, KIM
M26	Muara Angke, North Jakarta	Leaf flakes	Conservation	2017	JW, KIM
M27	Muara Angke, North Jakarta	Soil	Conservation	2017	JW, KIM
M28	Muara Angke, North Jakarta	Soil	Conservation	2017	JW, KIM
M29	Muara Angke, North Jakarta	Soil	Conservation	2017	JW, KIM
M39	Muara Tawar, Bekasi	Sediment	Industry	2018	SO
M40	Muara Tawar, Bekasi	Sediment	Industry	2018	SO
M41	Muara Tawar, Bekasi	Leaf flakes	Industry	2018	SO
M42	Muara Tawar, Bekasi	Sediment	Industry	2018	SO
M43	Muara Tawar, Bekasi	Seaweed	Industry	2018	SO
M44	Muara Angke, North Jakarta	Sediment	Conservation	2018	SO
M45	Muara Angke, North Jakarta	Leaf flakes	Conservation	2018	SO
M46	Muara Angke, North Jakarta	Sediment	Conservation	2018	SO
M47	Muara Angke, North Jakarta	Leaf flakes	Conservation	2018	SO
M48	Mangrove Api Api, Yogyakarta	Sediment	Conservation	2018	SO
M49	Mangrove Api Api, Yogyakarta	Sediment	Conservation	2018	SO
M50	Mangrove Api Api, Yogyakarta	Sediment	Conservation	2018	SO
M51	Mangrove Api Api, Yogyakarta	Leaf flakes	Conservation	2018	SO
M52	Mangrove Api Api, Yogyakarta	Sand	Conservation	2018	SO
M53	Mangrove Api Api, Yogyakarta	Sediment	Conservation	2018	SO

CHAPTER 2. MATERIAL and METHODS

2.1. Material

2.1.1. Organisms

Twnety-seven of predatory bacterial isolates and 66 of the different type strains of *Myxococcales* used in this study were mentioned in Table 3 and Table 4, respectively.

Table 3. Predatory bacteria isolated from Indonesian Mangroves

No.	Strain	Mangrove	Species	Accession Number
1	455MSO	Muara Angke, Jakarta	*Archangium gephyra*	MW182273
2	82MSO	Muara Angke, Jakarta	*Corallococcus coraloides*	MW182281
3	412MSO	Muara Tawar, Bekasi	*Corallococcus coraloides*	MW182280
4	101MSO	Muara Angke, Jakarta	*Corallococcus coraloides*	MW182265
5	411MSO	Muara Tawar, Bekasi	*Myxococcus fulvus*	MW182283
6	191MSO	Muara Angke, Jakarta	*Myxococcus fulvus*	MW182272
7	35MSO	Muara Angke, Jakarta	*Myxococcus fulvus*	MW182276
8	511MSO	Mangrove Api-Api, Yogyakarta	*Myxococcus fulvus*	MW182268
9	483MSO	Mangrove Api-Api, Yogyakarta	*Myxococcus fulvus*	MW182269
10	471MSO	Muara Angke, Jakarta	*Myxococcus macrosporus*	MW182285
11	161MSO	Muara Angke, Jakarta	*Myxococcus macrosporus*	MW182282
12	451MSO	Muara Angke, Jakarta	*Myxococcus macrosporus*	MW182288
13	521MSO	Mangrove Api-Api, Yogyakarta	*Myxococcus macrosporus*	MW182286
14	431MSO	Muara Tawar, Bekasi	*Myxococcus macrosporus*	MW182287
15	421MSO	Muara Tawar, Bekasi	*Myxococcus macrosporus*	MW182284

16	532MSO	Mangrove Api-Api, Yogyakarta	*Myxococcus macrosporus*	MW182267
17	21MSO	Muara Angke, Jakarta	*Myxococcus macrosporus*	MW182275
18	173MSO	Muara Angke, Jakarta	*Myxococcus macrosporus*	MW182271
19	16MSO	Muara Angke, Jakarta	*Nannocystis pusila*	MW182277
20	112MSO	Muara Angke, Jakarta	*Nannocystis pusila*	MW182278
21	182MSO	Muara Angke, Jakarta	*Nannocystis pusila*	MW182270
22	61MSO	Muara Angke, Jakarta	*Chondromyces pediculatus*	MW182274
23	41MSO	Muara Angke, Jakarta	*Chondromyces robustus*	MW199130
24	151MSO	Muara Angke, Jakarta	*Chondromyces robustus*	MW182279
25	503MSO	Mangrove Api-Api, Yogyakarta	*Racemicystis persica*	MW182266
26	313MSO	Muara Angke, Jakarta	*Ohtaekwangia kribbensis*	MT591272
27	314MSO	Muara Angke, Jakarta	*Ohtaekwangia kribbensis*	MT591273

Table 4. Sixty-six of the different *Myxococcales* type strains

Family	**Genus**	**Species**	**DSM**	**Accession Number**
Myxococcaceae	*Aggregicoccus*	*edonensis*	27872	KF914661
	Corallococcus	*coralloides*	2259	NR074852
		exiguous	14696	DQ768121
		macrosporus	14697	NR042331
	Myxococcus	*fulvus*	16525	NR043946
		stipitatus	14675	DQ768118
		virescens	2260	NR043946
		xanthus	16526	DQ768116
	Pyxidicoccus	*fallax*	14698	DQ768123
Cystobacteraceae	*Archangium*	*gephyra*	2261	DQ768106
		disciforme	52716	NR117460
	Cystobacter	*armeniaca*	14710	DQ768107
		badius	14723	DQ768108
		ferrugineus	14716	AJ233901
		fuscus	2262	DQ768109

		gracilis	14753	DQ768110
		miniatus	14712	DQ768111
		minus	14751	AJ233903
		velatus	14718	DQ768115
		violaceum	14727	DQ768114
	Hyalangium	*minutum*	14724	DQ768124
	Melittangium	*boletus*	14713	AJ233908
		lichenicola	2275	DQ768126
	Stigmatella	*aurantiaca*	17044	GU207882
		erecta	16858	AJ970180
		hybrida	14722	DQ768129
		armeniaca	14710	DQ768107
Vulgatibacteraceae	*Vulgatibacter*	*incomptus*	27710	AB847448
Anaeromyxobacteraceae	*Anaeromyxobacter*	*dehalogenans*	21875	AF382396
	Minicystis	*rosea*	24000	GU249616
	Aetherobacter	*fasciculatus*	24601	GU249609
		rufus	24628	GU249610
	Byssovorax	*cruenta*	14553	AJ833647
	Chondromyces	*apiculatus*	14605	AJ233938
		crocatus	14714	GU207874
		lanuginosus	14631	AJ233939
		pediculatus	14607	GU207875
		robustus	14608	AJ233942
	Jahnella	*thaxteri*	14626	NR117461
	Polyangium	*sorediatum*	14670	GU207880
		fumosum	14668	GU207879
		spumosum	14734	GU207881
	Racemicystis	*crocea*	100773	KT591707
		iranensis	103165	KX443485
	Sorangium	*cellulosum*	14627	NR116678
		ambruticinum	53252	MG824979
		arenae	105768	MG824983
		bulgaricum	53339	GB824980
		dawidii	105767	MG824981
		kenyense	105741	MG824982
		orientale	105742	MG824978
		reichenbachii	105769	MG824984
Sandaracinaceae	*Sandaracinus*	*amylolyticus*	53668	HQ540311
Phaselicystidaceae	*Phaselicystis*	*flava*	21295	EU545827
Labilitrichaceae	*Labilithrix*	*luteola*	27648	NR126182
Nannocystaceae	*Enhygromyxa*	*salina*	15217	NR024807
	Nannocystis	*exedens*	71	M94279
		pusilla	14622	NR117463

		konarekensis	104509	KY381122
	Plesiocystis	*pacifica*	14875	NR024795
	Pseudenhygromyxa	*salsuginis*	21377	AB600195
Kofleriaceae	*Kofleria*	*flava*	14601	AJ233944
	Haliangium	*ochraceum*	14365	AB016470
		tepidum	14436	AB062751

2.1.2. Chemicals

Isolation and cultivation media for predatory bacteria, primer of 16S rRNA gene, primer of MLSA experiment and KIT's in this study were mentioned in Table 5, Table 6, Table 7 and Table 8, respectively.

Table 5. Information regarding the media for isolation and cultivation

Media	Composition		Notes
VY/2-Agar	10 mL/L	Baker's yeast*	pH 7.4
	0.10 %	$CaCl_2$.$2H_2O$	
	0.5 mg/L	Vitamin B12	
	0.10 %	$MgSO_4$ · 7H2O	
	50 mM	HEPES (11,9g/L)	
	1.5 %	Agar (Difco)	
*Baker's yeast	50 g/100 mL		pH 7.2
Water-Agar	0.15 %	$CaCl_2$.$2H_2O$	pH 7.2
	0.15 %	$MgSO_4$ · 7H2O	
	1 mL/L	Vitamin solution (Schlegel)*	
	1.5 %	Agar (Difco)	
*Vitamin Solution	0.2 %	Biotin	
	2%	Nicotin acid	
	1%	Thiamin	
	1%	4-aminobenzoeacid	
	0.5%	Pantothenant	
	5%	Pyridoxamin	
	2%	Cyanocobalamin	
ST21-Agar*	0.10 %	K_2HPO_4	2/3 Solution A (v/v)
	1 %	Baker yeast	
	1 %	Agar (Difco)	

	0.1 %	KNO_3	1/3 Solution B (v/v)
	0.1 %	$MgSO_4 \cdot 7H2O$	
	0.1 %	$CaCl_2 .2H_2O$	
	0.02 %	FeCl3	
	0.01 %	MnSO4.7H2O	

*Solution A + B were mixed after autoclave separately, and added 1 mL/L trace element

E-Agar	0.40 %	skim milk	pH 7.4
	0.40 %	soy flour (degreased)	
	0.20 %	yeast extract (Marcor type 9000)	
	1 %	starch (Cerestar)	
	0.10 %	MgSO4 · 7H2O	
	50 mM	HEPES (11,9g/l)	
	8 mg/L	Fe-EDTA	
	0.50 %	glycerol (87% w/v)	
R2A	0.5 g	yeast extract	pH 7.2
	0.5 g	proteose peptone (Difco no. 3)	
	0.5 g	Casamino acids	
	0.5 g	Glucose	
	0.5 g	Soluble starch	
	0.30 g	Na-pyruvate	
	0.30 g	K2HPO4	
	0.05 g	MgSO4 · 7H2O	
	15 g	Agar	

Table 6. Information regarding the primer of 16S rRNA gene.*Position number refers to the 16S sequence of E. coli rrnB (GenBank J01695)[50].

Primer	**Sequence (5'-3')**	**Position***
F27	AGA GTT TGA TCM TGG CTC AG	8-27
R518	CGT ATT ACC GCG GCT GCT GG	518-537

F1100	YAA CGA GCG CAA CCC	1100-1114
R1100	GGG TTG CGC TCG TTG	1100-1114
R1525	AAG GAG GTG ATC CAG CCG CA	1522-1541

Table 7. Information regarding the primer for *Myxococcales* strains amplification

Primers covering coding sequence of **gyrB** (the β-subunit of DNA gyrase), **lepA** (leader peptidase, GTP binding membrane protein), **fusA** (fusidic acid resistance, protein sequence elongation factor G), **rpoB** (the β-subunit of RNA polymerase), **pyrG** (the cytidine triphosphate synthetase) and **pgm** (phosphoglucomutase) were used in our study.

Locus	**Primer**	**5'-3'**	**Amplicon size (bp)**	**Annealing temperature**	**Suborder**
gyrB	gyrBBAUP2	GCG GAA GCG GCC NGS NAT GTA	1300	60	*Cystobacterineae*
	gyrBBNDN1	CCG TCC ACG TCG GCR TCN GYC AT		66.9	*Sorangiineae / Nannocystineae*
lepA	lepABAUP1	CAT CGC CCA CAT CGA YCA YGG NAA	915	60	All of suborder
	lepABIDN1	CAT GTG CAG CAG GCC NAR RAA NCC			
fusA	fusA-F	CAT CGG CAT CAT GGC NCA YAT HGA	723	60	*Cystobacterineae*
	fusA-R	CAG CAT CGG CTG CAY NCC YTT RTT		57.1	*Sorangiineae / Nannocystineae*
rpoB	rpoB- F	GCG ATC AAG GAG CGC ATG AG	1257	60	All of suborder

	rpoB-R	CCA CGG CAT GAA CGC GAC			
pyrG	pyrG-F	GAY CCS TAC ATC AAY GTS GAY	435	60	All of suborder
	pyrG-R	GTG CTGS GTG GGC TTS GTC TT			
Pgm	pgm-F	CAT CTC SCA CGC SAT CCT C	1106	60	All of suborder
	Pgm-R	AAG CTC TCC GCG TAG ATY TTG TAG A			

Table 8. Information regarding the KITs with purpose in this study

Kits	Manufacturer	Catalog Number	Purpose
Invisorb® Spin Plant Mini Kit	Stratec Molecular	1037100300	Isolation and purification of total DNA from a wide variety of plant / tissue / cells
MOBIO PowerSoil® DNA Kit	Qiagen	12888-50	For the isolation of microbial genomic DNA from all soil types
NucleoSpin® Gel and PCR Clean-up	Macherey-Nagel	740.609.250	Two in one kit for PCR clean up and gel extraction
API® ZYM	BioMérieux	95060-706	Semiquantitation of enzymatic activities
API® CAMPY	BioMérieux	95060-690	API gram negative identification, 24-hour identification of *Campylobacter* species
Gen III Microplate Biolog System	Biolog	1030 / 72401	"Phenotypic Fingerprint" of the microorganism that can be used to identify it at the species level.

2.1.3. Equipment

Table 9. Information regarding the equipment in this study

Equipment	Manufacturer
Centrifuge	Eppendorf Centrifuge 5804 R
Centrifuge	Eppendorf Centrifuge 5427 R
Clean Bench	Thermo Scientific Type MS 2020 1.2
HPLC	Agilent 1260 Series; Aligent technology, USA
HPLC	Aligent 1100 series; Aligent technology, USA
HPLC column	XBrigde® C-18 3.5 µm, 2.1 mm x 100 mm, Waters
MS (HRESIMS)	MaXis ESI-TOF-MS spectrometer (Bruker) equipped with an Agilent 1260 series RP-HPLC system
N2 dryer (plates)	MiniVap (porvair science)
Nano-Photometer	IMPLEN Nano Photometer UV / VIS Spektralphotometer
Rotary evaporator	Heidolph Laborata 4003
Shaker	Pilot-Shake System Kühner RC-6-U
Shaker (plates)	Heidolph Titramax 1000
Thermocycler	Eppendorf Thermocycler Mastercycler gradient

2.2. Methods

Evaluation of myxobacterial diversity by Illumina Miseq Sequencing

2.2.1. DNA extraction

Six samples M39, M41, M44, M45, M48 and M52 (Table 2) were extracted according to a modified protocol of MOBio PowerSoil® DNA Kit (Qiagen). 0.25 g of the samples were mixed with 60 µL of Solution C1 (Table A1) in a Power-Bead tube and vortexed twice at maximum speed for 10 min. The mixture was centrifuged at 10,000 g for 1 min. After removal of the supernatant, the pellet was further proceeded through DNA binding and elution steps following the manufacturer's protocol. The quality of the DNA was assessed by 2 % agarose gel electrophoresis (Biorad Power Pac 300, 100 Volt for 30 min) and measured in Nano-photometer IMPLAN.

2.2.2. Amplicon library preparation

Amplification was performed using PrimerSTAR HS DNA Polymerase (Takara, Otsu, Shigu, Japan) following the manufacturer's instructions. Amplification methods were used as described by Rath *et al.* [51]. Modified forward primers W2 and W5 [31] specifically targeting *Cystobacterineae* and *Sorangiineae*/*Nannocystineae* of the *Myxococcales* were separately used in conjunction with reverse primer R1525 [36, 37] in a first PCR reaction. Samples were pre-denaturation at 96°C for 3 min following twenty-five cycles of denaturation at 94°C for 1 min, annealing at 56.6°C for 1 min, extension at 72 °C for 2 min. The PCR product was checked on 2.0 % agarose gel electrophoresis and purified using NucleoSpin Gel PCR Clean up Kit (Macherey-Nagel, Düren, Germany). One microliter of cleaned PCR product was used as template in a second PCR with primers 807F and 1050R containing part of the sequencing primer sites as short overhangs (Table A2) for 20 cycles to enrich for target sequences. A third amplification step of 10 cycles added the two indices and Illumina adapters to amplicons [51]. Obtained products were pooled in equimolar ratios and sequenced on an Illumina Miseq (2x300 bases, San Diego, USA).

2.2.3. Data analysis

The bioinformatics processing was performed as previously described [52]. Raw reads were merged with the Ribosomal Database Project (RDP) assembler [53]. Sequences were aligned within MOTHUR [54] (gotoh algorithm using the SILVA database [55]) and subjected to preclustering (diffs=2) yielding so-called phylotypes that were filtered for an average abundance of $\geq 0.001\%$ and a sequence length ≥ 250 bp before analysis. Phylotypes were assigned to a taxonomic affiliation based on the naive Bayesian classification with a pseudo-bootstrap threshold of 80% [53, 56]. The potential duplications of same *Myxococcales* sequences between both of set primers pair were counted and should be included to each of primer pair analysis. All sequences not matching to the *Myxococcales* order were deleted before further analysis. The relative abundance of genera was plotted using Microsoft

Excel._Sequences were deposited at NCBI database under accession number in Table S1.

Potential of myxobacteria from Indonesia mangroves as antimicrobial agents

2.2.4. Isolation of Myxobacteria

Twenty-five strains were isolated according to an established procedure by Mohr *et al.* [36, 37]. After five to seven days incubation, swarming colonies and or fruiting bodies were observed under a dissecting microscope (Olympus SZX10). A light microscope (Zeiss Axio Scope. A1. Microscope) was used for definition of the purify strains by single uniform cells. Each pure culture from VY/2 agar medium [3, 57] was transferred into a flask containing 20 mL CY/H broth medium [3, 57] as a seed culture. 10 mL of the seed culture were scaled up into 100 mL of CY/H broth medium and portions of 1.5 mL of cell mass cultures were directly stored at -80 ^{o}C.

2.2.5. Identification of pure cultures by 16S rRNA gene sequencing

One mL from 100 mL of well-grown cultures in CY/H broth medium was taken and centrifuged (Eppendorf Centrifuge 5427R) at 11,000 rpm for 5 min. After removal of the supernatant, the pellet was used for DNA extraction following the manufacturer's protocol from Invisorb Spin Plant mini kit (Stratec Molecular, Germany).

After the genomic DNA was extracted, the 16S rRNA sequences were amplified with primer pair F27/R1525 according to the procedure by Mohr *et al*. [36, 37]. PCR products were examined by agarose gel electrophoresis (0.8 %) (Biorad Power Pac 300, 70 Volt for 40 min) and purified following the manufacturer's protocol of NucleoSpin Gel and PCR Clean up Kit (Macherey-Nagel, Düren, Germany).

The forward and reverse sequences of 16S rRNA gene fragments were assembled with the BioEdit program [58] and closely related type strains were identified using

the NCBI 16S rRNA gene database [59] or Ribosomal Database Project (RDP) [53].

Phylogenetic tree was calculated using the DSMZ phylogeny service for the application of phylum-specific 16S similarity thresholds [60, 61]. A multiple sequence alignment was created with MUSCLE [62]. Maximum likelihood (ML) trees were inferred from the alignment with RAxML [63] and rapid bootstrapping in conjunction with the autoMRE bootstrapping criterion [64]. One-thousand bootstrapping replicates were used in conjunction with tree-bisection-and-reconnection branch swapping and ten random sequences addition replicates. Finally, the sequences were checked for a compositional bias using the X^2 test as implemented in PAUP* [65].

2.2.6. Preparation of crude extract

100 mL of well-grown cultures were prepared in CY/H broth medium. Ten % of the well-grown cultures were transferred into 250 mL shake flasks containing 100 mL of different media (Table A3) with 2 % absorber resin Amberlite XAD-16. The flasks were incubated on a rotary shaker with 160 rpm at 30 °C for 7 up to 14 days (depending on the growth of myxobacteria). The XAD-resin was separated from the supernatant by sieving, extracted directly with 70 mL acetone. The acetone extract was evaporated to dryness and the residue was dissolved in 1 mL methanol and stored at -20 °C.

2.2.7. Preparation of pathogenic microorganisms

Pathogenic microorganisms were obtained from Microbial Strain Collection Group, HZI Germany. The selected pathogenic microorganisms used in antimicrobial study were Gram-negative bacteria *Escherichia coli* WT-BW 25113, *Escherichia coli* JW0451-02, *Acinetobacteria baumanii* DSM 30008, *Pseudomonas aeruginosa* Pa14, Gram-positive bacteria *Staphylococcus aureus* Newmann, *Citrobacter freundii* DSM 30039, *Mycobacter smegmatis* ATCC 700084, yeast

Wickerhamomyces anomalus DSM 6766, *Candida albicans* DSM 1665 and filamentous fungus *Mucor himalis* DSM 2656 (Table A4).

2.2.8. Screening for antimicrobial activity and identified of active compound

The antimicrobial activity was assessed by a growth inhibition test using a serial dilution of each crude extract against different pathogenic microorganisms in a 96-well plate [66]. Minimum inhibitory concentrations (MIC) in µg/mL were determined by serial dilution in 96-well plates with Mueller Hinton (MH) [67] and MYC [68] medium for bacteria and yeast/fungi, respectively. The pathogenic microorganisms were inoculated into 20 mL medium (Table A3) and 280 µL of each were distributed from the first row using a multichannel pipette (RAININ 8-Kanal-Pipette ED P3 Plus 100-1200 µL) followed by 150 µL for the next row. Twenty microliter aliquots (150 µg/mL from 1 mg/mL) of crude extracts were tested against ten different pathogenic microorganisms as mentioned above [66]. The negative control wells were left blank. Cell density of the pathogenic microorganisms was (OD600): 0.01 (v/v) for bacteria and 0.05 (v/v) for fungi.

The 96-well plates were incubated on Heidolph Titramax 1000 shaker with 750-rpm at 30 ^{o}C or 37 ^{o}C for 24-48 hours (Table A4). The wells with clear zones indicated the inhibition of growth of the pathogenic microorganisms by the crude extracts whereas high cell density was observed in the positive control.

Crude extracts showing activity in 96-well plates were fractionated with bioassay-guided screening using analytical HPLC combined with diodearray-UV spectrometry (The parameter condition mentioned in Table A5 (Agilent 1260 Series; Agilent technology USA) for peak-activity correlation [22]. After evaporation of the HPLC eluent, the wells were inoculated with 150 µL (each well) of the previously tested pathogenic microorganism and incubated as mentioned above.

Active crude extracts were characterized and identified using Data Analysis 4.2 b383 (Bruker Daltonics) Krug [69] and Hoffmann [14] and in-house SQL

myxobase database. The antimicrobial activity was visualized quantitatively by heat map with Heatmapper software [70].

Multilocus sequences analysis (MLSA) of myxobacteria from mangrove

2.2.9. *Myxococcales* strains

Sixty-six of the different type strains of *Myxococcales* (Table 4) from the Microbial Strain Collection of the Helmholtz Center for Infection Research, Germany and 13 *Myxococcales* strains from Indonesian mangroves (Table 3) were used in this study. The GenBank/EMBL/DDBJ accession numbers for the housekeeping genes sequences of the strains were mentioned in Table S3.

2.2.10. Cultivation and DNA extraction

The methods are already mentioned in 2.2.5.

2.2.11. Amplification and Sequencing

DNA extraction was performed following manufacturer's protocol from Invisorb Spin Plant Mini Kit (Stractec Molecular, Germany). DNA amplification varied considerably and required different parameters among the members of *Myxococcales* (Table 7). For the suborder *Cystobacterineae,* the genomic DNA was amplified using touchdown PCR (Eppendorf Mastercycler Gradient) [72], whereas for the suborder *Sorangiineae/Nannocystineae,* the genomic DNA was amplified following the protocol by Mohr *et al*. [37]. The PCR product was purified following the manufacturer's instruction by NucleoSpin Gel and PCR Clean up Kit (Macherey-Nagel, Düren, Germany) and sequenced in the working group Genome Analytics (HZI, Braunschweig, Germany).

2.2.12. Data analysis

All gene sequences were trimmed, assembled and aligned using BioEdit Sequence Alignment software [58]. The ranges of similarities of 16S rRNA gene and concatenated gene sequences were analyzed using MEGA X software [73]. Analysis of DNA sequence variations of 16S rRNA gene, single housekeeping gene, and concatenated housekeeping genes, such as number of allele and parsimony informative sites, the number of segregating sites (S), allelic diversity, and the nucleotide diversity (Pi) mean G+C content (mol %) were analyzed using the software DnaSP version 5.10 (http://www.ub.edu/dnasp/) [74]. The phylogenetic tree was performed using the DSMZ phylogeny service as mentioned in 2.2.2.2.

Ohtaekwangia **sp. nov., a gliding bacterium isolated from Indonesian mangroves and the related strains from HZI strain collection**

2.2.13. Isolation and Identification of Gliding Bacteria

The methods for isolation and identification of gliding bacteria are mentioned in 2.2.1. Phylogenetic tree analysis inferred from the 16S rRNA gene sequence is mentioned in 2.2.2.

2.2.14. Preparation of cultures and type strains

The candidate of a new species was re-activated from cryopreservation in 10 mL of VY/2 liquid medium. After five days incubation at 30 °C, 10 mL of the well-grown cultures were transferred into 100 mL of VY/2 medium. The well-grown cultures were further used for characterization of strain 313MSO. *Ohtaekwangia koreensis* 3B-2 DSM 25262^{T} and *Ohtaekwangia kribbensis* 10A0 DSM 25221^{T} were used in this study for comparison of strain 313MSO. Using the same procedure as mentioned above, the type strains were re-activated in R2A medium, which is the standard medium as recommended by DSMZ.

2.2.15. Morphological analysis

2.2.15.1. Morphology

The gliding move of colonies on the VY/2 agar plate was traced under a dissecting microscope (Olympus SZX12) and recorded with an AxioCam MRc camera. The morphological characteristics of cells were examined using the well-grown culture mentioned in section 2.2.14. One-hundred µL of 100 mL of the well-grown culture were centrifuged at 11,000 rpm for 8 min. The pellet was washed twice with 1 mL deionized water followed by centrifugation at 11,000 rpm for 8 min and 20 µL of 100 µL pellet were spread on a slide. The morphology and cell size were observed using light microscope (Zeiss Axio Scope. A1. Microscope) at a magnification 1,000x. The average of cell length was measured using Axio-Vision Rel. 4.8 software.

2.2.15.2. Gram Staining

After 20 µL of cell suspension were spread on a slide as mentioned in section 5.1.3.1, the Gram staining was prepared following the protocol by Sigma Aldrich Gram Staining Kit (77730). It was observed under a light microscope (Zeiss Axio Scope. A1. Microscope) with a magnification of 1,000x.

2.2.15.3. Physiological analysis

2.2.15.3.1. Medium variation

Medium variation is a first polyphasic approach for characterization of new strain. Four different agar-media R2A, VY/2, CY and VY/4 ASW were selected for growth characterization. After five days of incubation at 30 °C, the plates were checked by visual inspection.

2.2.15.3.2. Detection of optimal temperature for growth

Ten µL of 100 mL of a well-grown culture were inoculated with VY/2 agar-medium, then incubated at different temperatures (10 °C, 20 °C, 25 °C, 28 °C, 30 °C, 34 °C, 40 °C and 44 °C)[75]. After five days of incubation, the plates were

checked by visual inspection. The optimal temperature was defined by swarming colonies on plates.

2.2.15.3.3. Detection of optimal pH for growth

Medium was prepared with different pHs 4; 5; 6.5; 7.5; 8.5 and 9 [75]. For acidic and basic conditions, pH values were adjusted with 1 % HCl (v/v) and 4.5 % NaOH (v/v), respectively. Ten µL of 100 mL of a well-grown culture were inoculated with VY/2 agar-medium at different pHs and incubated at the optimal temperature condition (see section 2.2.15.3.2). The optimal pH was defined by swarming colonies on plates after five days incubation.

2.2.15.3.4. NaCl Tolerances

In the case of halophilic representatives, 10 µL of 100 mL of a well-grown culture were inoculated with VY/2 agar-medium at the optimal pH value of new isolate in different concentrations of 0; 2; 5; 7.5 and 10 % NaCl (v/v) [75]. In addition, incubated at the optimal temperature condition of new isolate (see section 2.2.4.3.3.2) for five days. Strains are proven to be salt tolerant if swarming colonies were observed.

2.2.15.3.5. Anaerobic Tolerances

For detection of growth in an anaerobic atmosphere, an anaerocult reagent (Merck) was added in an anaerobic chamber together with 35 mL of distilled water [75]. An indicator oxygen strip was placed in the chamber. Ten µL of 100 mL of a well-grown culture were inoculated with VY/2 agar-medium at the optimal pH value 6.5-7.5 and incubated at the optimal temperature of 30 °C for fifteen days in the chamber. When swarming colonies were observed, strains are proven being tolerant to anaerobe conditions.

2.2.15.3.6. Catalase and Oxidase Test

A well-grown culture in VY/2 agar medium was prepared. Catalase- and Oxidase-tests were carried out following manufacturer instruction of Sigma Aldrich. A

positive result for catalase and oxidase activity was indicated by bubble formation and discoloration to purple-blue after 1 min incubation, respectively.

2.2.15.3.7. Flexirubin Pigment Test

Flexirubin type pigments play an important role within the members of *Cytophagales* [17]. Ten µL of the pellet (see 2.2.15.1, mixed with 1 mL deionized water) were mixed with 1 drop of 20 % KOH (v/v) on a slide glass and a positive result was performed by discoloration to red-brown.

2.2.15.3.8. APIZYM and APICAMPY Test

Enzyme profiles were performed using APIZYM [76] and APICAMPY [77] strip tests following manufacturer's protocol. List of the substrate for enzyme reaction were mentioned in Table A6.

2.2.15.3.9. Gen III Microplate Biolog System

Assimilation for various substrates of new strain was evaluated using Gen III Microplate Biolog System following manufacturer's protocol. List of the assimilation for various were mentioned in Table A7.

2.2.15.3.10. Antibiotic Resistances

A panel of antibiotics were prepared together on VY/2 medium plates (Table 10). Antibiotic sensitivity was evaluated following disc-diffusion plate method [78].

Table 10. List of the antibiotics for characterization of new strain

No.	Antibiotic	Concentrations (mg/mL)
1	Polymyxin	10
2	Gentamicin	10
3	Oxytetracyclin	10

4	Ampicilin	10
5	Chloramphenicol	9
6	Spectinomycin	10
7	Kanamycin	10
8	Cephalosporine	10
9	Fusidin acid	10
10	Bacitracin	10
11	Thiostrepton	10
12	Trimethoprim	5
13	Hygromycin	50

2.2.15.4. Chemotaxonomy

2.2.15.4.1. Polar lipid Analysis

Lyophilized cells were carried out in the first step of polar lipid analysis. Two-hundred mL of a well-grown culture were centrifuged at 9,000 rpm for 10 min. The sediment was washed with deionized water three times and centrifuged at 9,000 rpm for 10 min. The residue was lyophilized and stored at room temperature. Polar lipids from lyophilized cells were extracted following the protocol by Minnikin *et al*. [79] and identified as described by Collin and Shah [80]. Phospholipids were observed by visual inspection that showed different spots on plates and were defined using standard phospholipids on Table 11.

Table 11. Phospholipid Analyses

Plate	Reagent	Temp (°C)	Time (min)	Defined as	Colors Spot
1	Molybdat-phosphoric-acid	150	5	Total lipid	Blue
2	Ninhydrin	100	5	Amino group	Red
	Molibdan blue	120	5	Phospholipid	Blue
	Without reagent	150	7	All organic	Brown
3	Alpha-naphthol-sulfuric acid	120	5	Sugary lipid	Red / Blue
4	Dragendorff reagent	-	-	Nitrogen compound	Yellow oranges
5	Anisaldehyde	100	10	Sugary lipid	Violet/green

2.2.15.4.2. Fatty Acid Methyl Ester (FAME)

Fatty acid methyl ester was extracted following the protocol of Microbial Identification System (MIDI) and analysed by GC-MS.

CHAPTER 3. RESULTS

3.1. Evaluation of myxobacterial diversity by Illumina Miseq Sequencing

Two set of primer pairs W2/R1525 and W5/R1525 were selected as potential specific primers for myxobacterial diversity analyzed by the NGS method. A total of 20,057 myxobacterial sequences (1761±1002 SEM per sample) from 12 samples, where each sample was amplified twice with the two different primer pairs were obtained. Table 12 summarizes the W2/R1525 primers that are used successfully in pair to amplifiy members of the *Myxococcales* order between 0.56 % and 70.42 % of the obtained reads. With the W2/R1525 primer pair, 7.27-27.03 % of the amplified sequences were belonged to *Myxococcales*, indicating a low abundance of those bacteria in that sample. In contrast to the W5/R1525 primer pair, 70.42 % of *Myxococcales* sequences with the W2/R1525 primer pair in one Yogyakarta mangrove sample indicates high abundance of *Myxococcales*.

Thirteen major genera could be identified from three sampling sites analyzed in this study (Figure 5). The W2/R1525 primer pair could amplify sequences indicating the presence of *Cystobacter*, *Myxococcus, Stigmatella, Archangium* and *Anaeromyxobacter* of the *Cystobacterineae.* However, also *Haliangium* sequences could be amplified by this primer pair. The W5/R1525 primer pair amplified sequence indicating the presence of members of the genera *Haliangium*, *Kloferia* and *Nannocystis* of the *Nannocystineae* and *Chondromyces*, *Labilithrix*, *Phaselicystis, Polyangium* and *Sandaracinus* of the *Sorangiineae.* In addition, some sequences of *Myxococcus, Stigmatella* and *Cystobacter* of the *Cystobacterineae* could be observed as being amplified by this primer.

Overall, the primers showed good specificity for their targets. Clear differences in myxobacterial diversity could be observed in the sampling sites. *Stigmatella* spp. could be observed exclusively in MK samples, Yogyakarta mangroves whereas *Nannocystis* spp. and *Labilithrix* spp. were present only in Jakarta and Yogyakarta mangroves. Only *Myxococcus* spp. were observed in all six samples, indicating that it is a common myxobacterium in mangroves samples.

Table 12. The number of total reads sequences of Myxococcales in each sample. * MT: Bekasi, MA: Jakarta, MK: Yogyakarta

Samples	**Total Bacteria Sequences in Sample**		***Myxococcales* Sequences in Sample**		**Percentage of *Myxococcales* in Sample (%)**		**Location***
	W2/R1525	**W5/R1525**	**W2/R1525**	**W5/R1525**	**W2/R1525**	**W5/R1525**	
M39	8819	4816	437	350	4.96	7.27	MT
M41	21064	10537	315	674	1.50	6.40	MT
M44	12053	3280	204	758	1.69	23.11	MA
M45	9991	6170	56	450	0.56	7.29	MA
M48	17520	5428	12338	1467	70.42	27.03	MK
M52	13007	10743	1446	1562	11.12	14.54	MK
Total	82454	40974	14796	5261	-	-	
Mean	-	-	-	-	15.04	14.27	

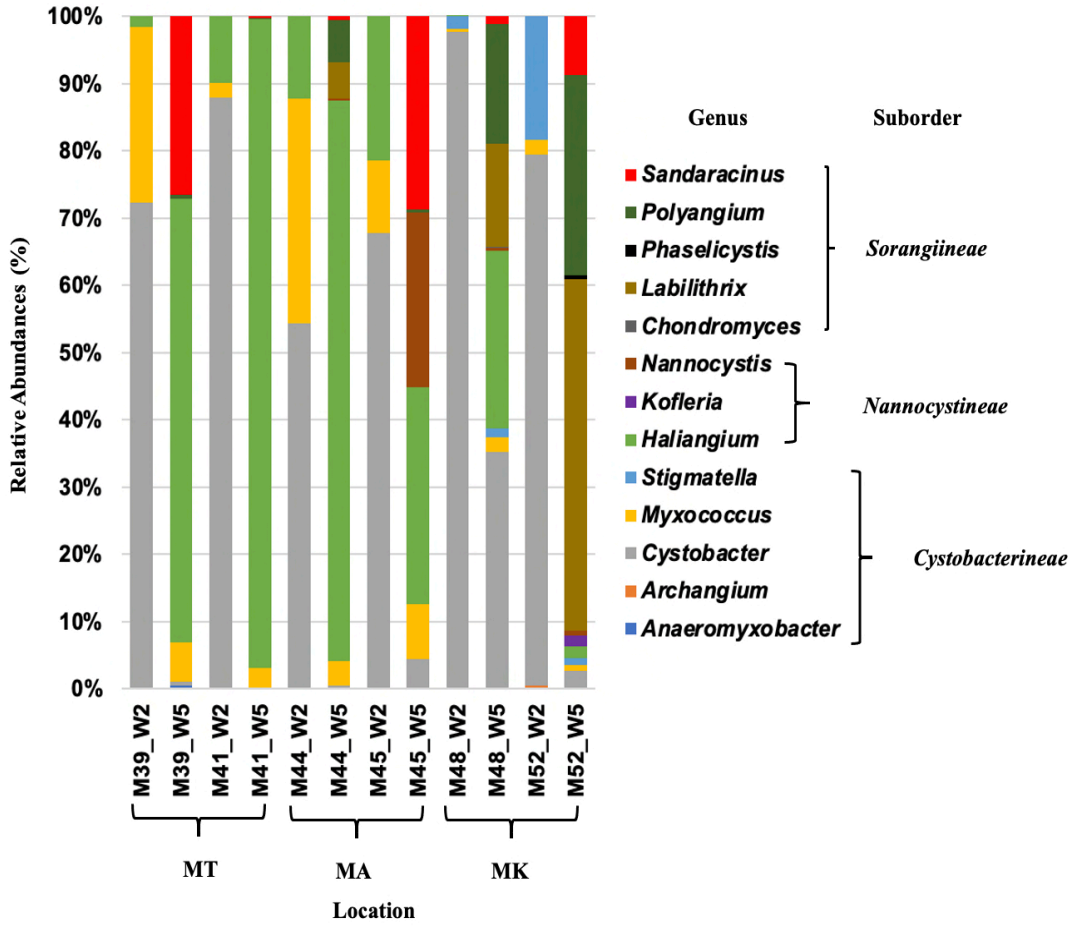


Figure 5. Relative abundances of myxobacterial genera in Indonesian Mangroves. MT: Bekasi MA: Jakarta MK: Yogyakarta using different set of primer W2/R1525 and W5/R1525. The community composition was revealed using primers targeting the suborder Cystobacterineae and the suborders Sorangiineae/Nannocystineae, respectively.

3.2. Potential of myxobacteria from Indonesia mangroves as antimicrobial agents

3.2.1. Cultivation of myxobacteria

Seventy myxobacterial isolates were successfully isolated from three mangrove sites. All of the strains showed fruiting body formation and swarming on a surface agar-medium. Therefore, based on different morphologies and 16S rRNA gene sequence analysis, the number of myxobacteria by identifying replicates from same

sources and location was reduced. Twenty-five isolates, 16 from MA sampling site, 3 from MT sampling site and 6 from MK sampling site were selected for further analysis. Table 13 summarizes the data of identities of myxobacterial isolates from mangroves based on partial 16S rRNA gene sequence analysis and a phylogenetic analysis of 16S rRNA gene sequences from the 25 isolates is shown in Figure *6*A. Some isolates showed less than 98.70% similarity to close related type strains. Therefore, full genome sequencing and a polyphasic comparison are needed for their further characterization and whether they comprise novel species.

The fruiting bodies forming as main characteristics of myxobacterial isolates, which previously described by Reichenbach *et al*. [4][16], can be seen in Figure 2. *Myxococcus* sp. strain 431MSO and 451MSO have spherical fruiting bodies with yellow or oranges red colors on VY/2 agar medium. *Coralococcus* sp. strain 412MSO makes swarm colonies and forms fruiting bodies with coralloid-branched shapes. *Chondromyces* sp. strain 151MSO builds tree shaped fruiting bodies on Stan21 agar medium and *Archangium* sp. strain 455MSO makes swarm colonies with branched radial veins on VY/2 agar medium. *Racemycistis* sp. strain 503MSO has swarming area like the *Sorangium* genus on VY/2 agar medium.

A phylogenetic analysis of 16S rRNA gene sequences from 25 myxobacterial isolates is shown in Figure *6*. These 25 isolates were grouped into three *Myxococcales* suborders: *Nannocystaceae*, *Sorangiineae* and *Cystobacterineae*. For *Nannocystaceae*, the level of similarity among *Nannocystis pusila* strains was 99.01 % to 99.56 %. For *Sorangiineae*, the level of similarity among *Chondromyces* spp. strains and *Racemicystis persica* were 95.34 % to 98.52 % and 97.98 %, respectively. For *Cystobacterineae*, the level of similarity to *Archangium gephyra* was 98.07 %, whereas among *Corallococcus coraloides* stains was 99.16 % to 99.66 %. Furthermore, the level of similarity among *Myxococcus* spp. strains was 97.66 % to 99.88 %.

Table 13. Identities of myxobacterial isolates from Indonesian mangroves based on 16S rRNA gene sequences.

* MT: Bekasi MA: Jakarta MK: Yogyakarta

No	Next Related Type Strain	Type Strain Accession Number	Sample Name	Sample Accession Number	Similarity to Type Strain (%)	Sequence Length (bp)	Sources	Location
	Suborder *Cystobacterineae*							
1	*Archangium gephyra* DSM2261^T	DQ768106	455MSO	MW182273	98.07	883	Leaf flakes	MA
2	*Corallococcus coraloides* DSM2259^T	NR074852	82MSO	MW182281	99.66	893	Soil	MA
3	*Corallococcus coraloides* DSM2259^T	NR074852	101MSO	MW182265	99.22	896	Soil	MA
4	*Corallococcus coraloides* DSM2259^T	NR074852	412MSO	MW182280	99.16	950	Leaf flakes	MT
5	*Myxococcus fulvus* DSM16525^T	NR043946	35MSO	MW182276	98.33	897	Soil	MA
6	*Myxococcus fulvus* DSM16525^T	NR043946	191MSO	MW182272	99.55	880	Soil	MA
7	*Myxococcus fulvus* DSM16525^T	NR043946	411MSO	MW182283	99.36	932	Soil	MT
8	*Myxococcus fulvus* DSM16525^T	NR043946	511MSO	MW182268	98.20	890	Leaf flakes	MK
9	*Myxococcus fulvus* DSM16525^T	NR043946	483MSO	MW182269	99.33	891	Soil	MK
10	*Myxococcus macrosporus* DSM14697^T	NR042331	471MSO	MW182285	99.88	860	Leaf flakes	MA
11	*Myxococcus macrosporus* DSM14697^T	NR042331	161MSO	MW182282	99.78	896	Leaf flakes	MA
12	*Myxococcus macrosporus* DSM14697^T	NR042331	451MSO	MW182288	99.34	916	Leaf flakes	MA
13	*Myxococcus macrosporus* DSM14697^T	NR042331	21MSO	MW182275	98.71	928	Soil	MA
14	*Myxococcus macrosporus* DSM14697^T	NR042331	173MSO	MW182271	98.76	885	Soil	MA
15	*Myxococcus macrosporus* DSM14697^T	NR042331	421MSO	MW182284	99.70	928	Soil	MT

16	*Myxococcus macrosporus* DSM14697^T	NR042331	521MSO	MW182286	99.10	893	Sandy Beach	MK
17	*Myxococcus macrosporus* DSM14697^T	NR042331	431MSO	MW182287	99.78	929	Seaweed	MK
18	*Myxococcus macrosporus* DSM14697^T	NR042331	532MSO	MW182267	97.66	992	Soil	MK
	Suborder *Sorangiineae*							
19	*Racemicystis crocea* DSM100773^T	KT591707	503MSO	MW182266	98.30	890	Soil	MK
20	*Chondromyces robustus* DSM14608^T	AJ233942	41MSO	MW199130	95.38	763	Leaf flakes	MA
21	*Chondromyces robustus* DSM14608^T	AJ233943	151MSO	MW182279	95.34	1225	Leaf flakes	MA
22	*Chondromyces pediculatus* DSM14607^T	GU207875	61MSO	MW182274	98.52	1012	Leaf flakes	MA
	Suborder *Nannocystineae*							
23	*Nannocystis pusila* DSM53154^T	NR117463	112MSO	MW182278	99.01	905	Soil	MA
24	*Nannocystis pusila* DSM53154^T	NR117463	182MSO	MW182270	99.56	899	Soil	MA
25	*Nannocystis pusila* DSM53154^T	NR117463	16MSO	MW182277	99.45	906	Leaf flakes	MA

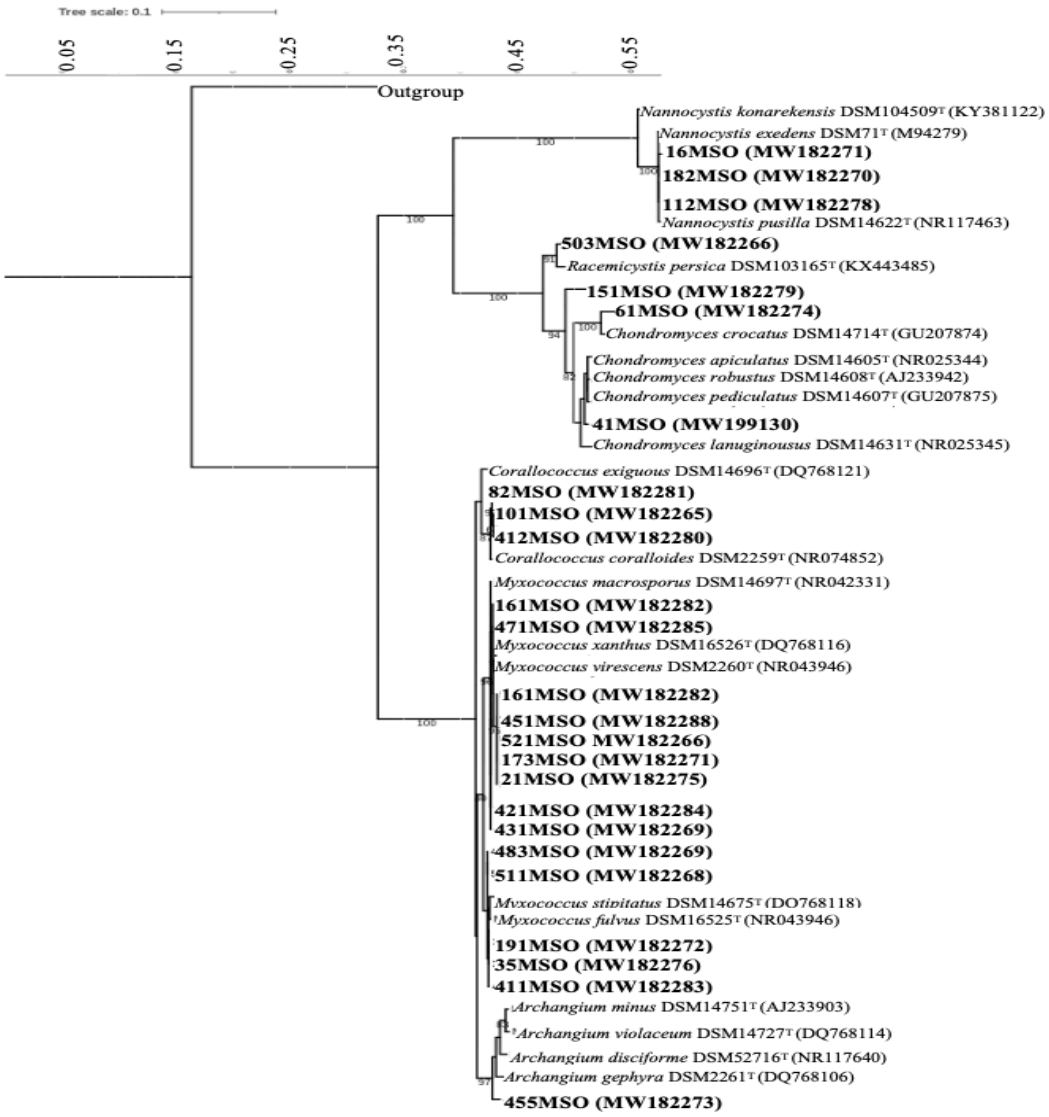


Figure 6. Phylogenetic tree of 25 myxobacterial strains isolated from Indonesian mangrove. Number above the branches are support values when larger than 60 % from ML (maximum likelihood) with respective 1000 bootstrap values.

3.2.2. Antimicrobial activities from cultivated of myxobacteria

Twenty-five crude extracts from 25 strains were screened for antimicrobial activities against a panel of human pathogenic microorganisms including five Gram-negative bacteria, two Gram-positive bacteria, yeast and fungi (Figure 7). Thirteen crude extracts showed potent activity against at least one of human pathogenic microorganisms. Two of them showed activity against both Gram-negative *Escherichia coli* strains as well as against *Citrobacter freundii* and nine

were active against Gram-positive *Staphylococcus aureus.* Furthermore, three of the extracts showed activity against the yeasts *Candida albicans* and *Wickerhamomyces anomalus.*

One out of thirteen active crude extracts from those myxobacteria, crude extract *Racemicystis* sp. strain 503MSO, which showed moderate activity against the yeast *Candida albicans* (Figure 7) was selected for further fractionation and compound identification. *Racemicystis* sp. strain 503MSO was interesting for further analyzes because the genus *Racemicystis* have a lack compound information in myxobase database yet and strain 503MSO was taxonomically characterized to may constitute novel species with partial 16S rRNA sequence similarity to type strains of ≤ 98.60 %.

Crude extract *Racemicystis* sp. strain 503MSO was fractionated in order to identify the responsible active compounds. The compound identification was done by comparing detected mass of the parent ions of the active fractions with in house myxobase database. The myxobase is a database to support research with myxobacteria, which are increasingly recognized as producers of secondary metabolites. Within myxobase, the information of bioactivity, retention time, UV spectrum, molecular mass and elemental formula of the molecule responsible for the active peak and HPLC chromatogram is provided. A summary of the active compounds from strain 503MSO is provided in Table 14, Figure S2.

Two active fractions from *Racemicystis* sp. strain 503MSO were identified comprising compounds with masses of *m/z* 375.2531 and 604.3857 $[M+H]^+$, respectively after the high-resolution mass-spectrometry (HR-MS) analysis of these active fractions. Based on comparison of its monoisotopic mass and retention time of compound described in Myxobase, the compound with *m/z* 375.2531 $[M+H]^+$ and 604.3847 $[M+H]^+$ could not be matched to any known compound in the database yet. Overall, this study confirmed that the neglected areas such as mangroves are promising habitats for isolation of novel myxobacterial strains and identification of unknown biological active compounds.

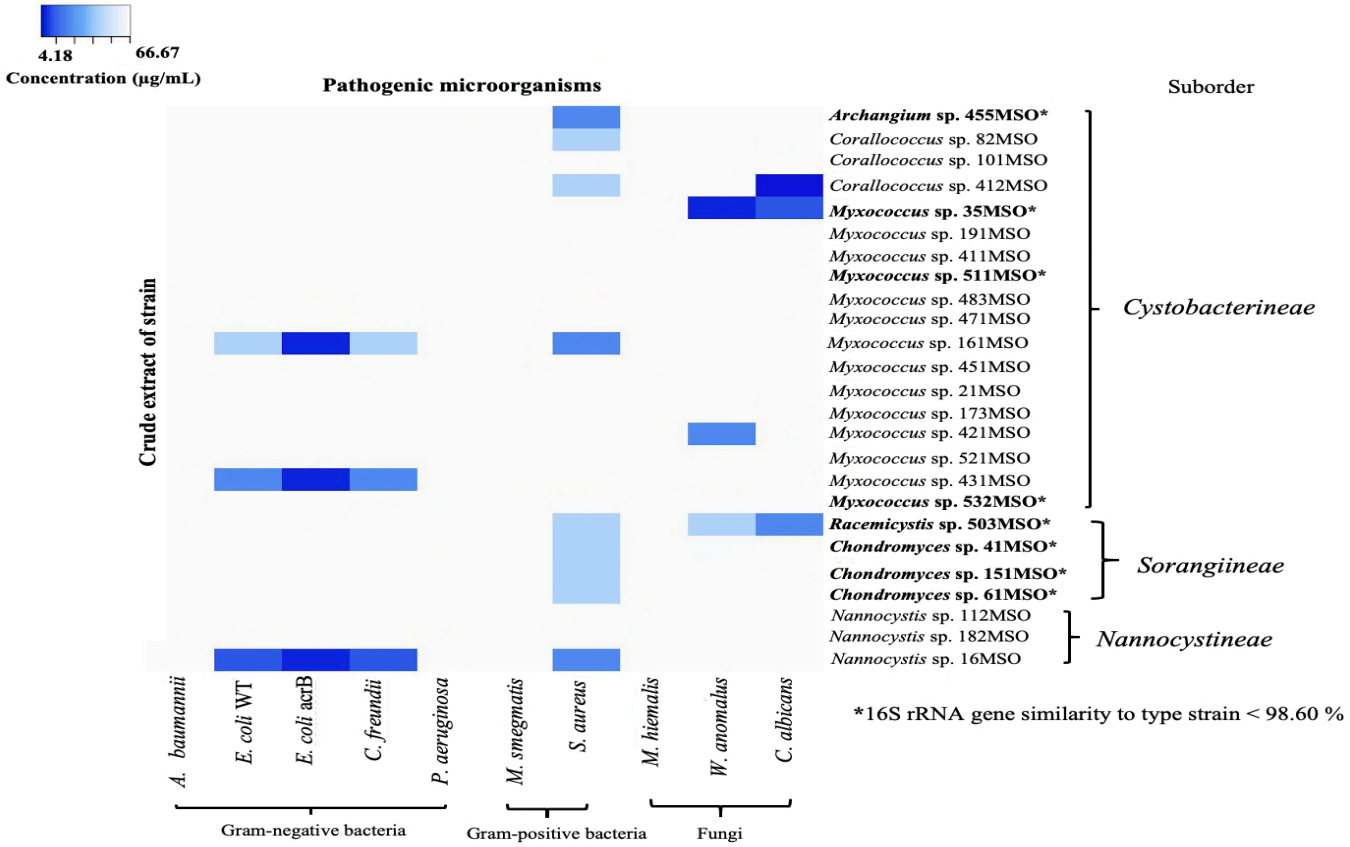


Figure 7**.** The antimicrobial activity of crude extracts from 25 myxobacterial isolates (Y-axis) acting against 10 different microbial pathogens (X-axis). The different colors indicate different minimum inhibitory concentrations (MIC) of crude extracts exerting inhibitory effects. MIC values were calculated by serial dilution with the highest concentration of 66.7 µg/mL (weak antimicrobial activity) diluted 8 times to the lowest concentration of 0.52 µg/mL (strong antimicrobial activity).

Table 3. Compounds identified with Myxobase[1] from extracts of isolated strain 503MSO.

Crude extract of / Compound name	In house SQL database of myxobase:				Experimental Data:			
	Mono isotopic Mass	Molecular Formula	RT (min)	Biological Source	Detected mass	Accurate mass	UV maxima (nm)	RT (min)
Unidentified	Unknown	Unknown	Unknown	Unknown	604.3847	603.3857	278	12.94
Unidentified	Unknown	Unknown	Unknown	Unknown	375.2531	374.2543	240	16.05

*RT is retention time. [1]Myxobase in-house database of myxobacteria group

3.3. Multilocus sequences analysis (MLSA) of myxobacteria from mangrove

3.3.1. Type strains

Almost all of the type strains were amplified with at least one of the housekeeping genes. However, three of housekeeping genes were not amplified with members of the suborder *Cystobacterineae* such as *Stigmatella aurantiaca* DSM17044, *Cystobacter fuscus* DSM2262, and *Archangium violaceum* DSM14727 (Figure 8). Consequently, the concatenated genes were not assembled for all of the type strains in this study and only 14 out of 66 type strains were selected for further study.

Table 13. Characteristics of three loci of 14 type strains. *Mean number of amplified nucleotides, including gaps; GC = Guanine + Cytosine content; S = number of segregating sites; π = nucleotide diversity.

Locus	Fragment size (bp)	Mean G+C content (%)	S	Phi	Parsimony informative sites		P-Distance	
					No.	%	Range	Mean
16S	1465	57	411	0.12122	315	21.5	0.0166-0.1879	0.097
fusA	846	65.4	435	0.3132	360	42.55	0.1082-0.4152	0.3195
gyrB	1684	67.3	676	0.29176	465	27.61	0-0.5147	0.2996
lepA	1108	66.7	598	0.36302	488	44.04	0.0449-0.5478	0.3885
Concatenated	3638	66.7	1709	0.32068	1313	36.09	0.0913-0.4803	0.3337

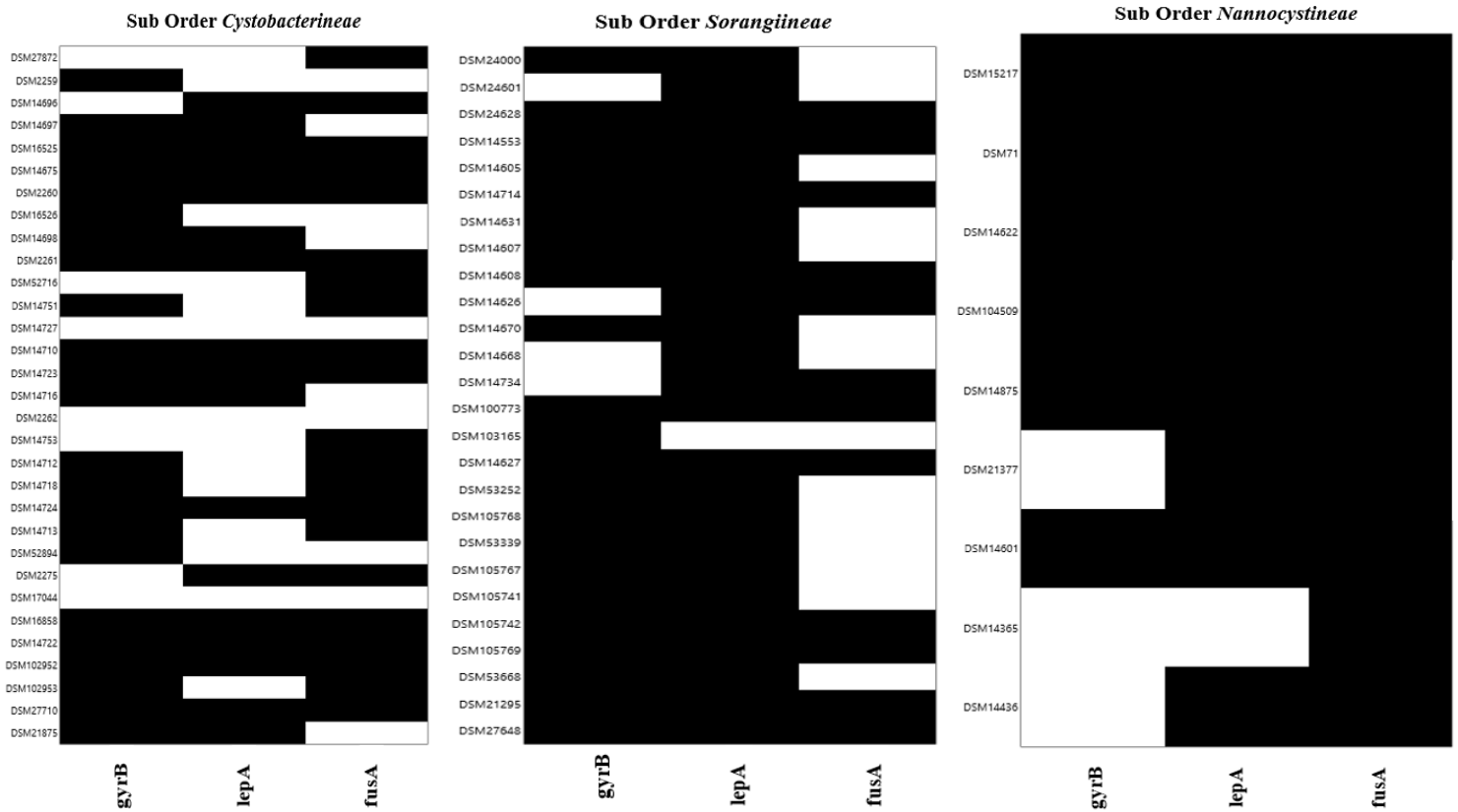


Figure 8**.** Amplified sequences result of three housekeeping genes from 66 type strains. In heat map, housekeeping genes, unamplified genes (white color) and amplified genes (black color).

Characteristics of housekeeping genes from 14 type strains are presented in Table 13. Multiple sequence alignment of the strains obtained higher fragment size of 3638 bp for concatenated gene *fusA-gyrB-lepA* than 16S rRNA gene (1465 bp). The guanine + cytosine content and p-distance of the housekeeping genes, and of the concatenated genes, respectively, were higher than those of the conserved 16S rRNA gene. It indicated that the distance of their phylogenetic position is wider than the distance of the conserved 16S rRNA gene.

3.3.2. Myxobacterial isolates

Although housekeeping genes are inconsistent to be amplified into type strains, six housekeeping genes from myxobacterial isolates were performed within *Myxococcales* strains (Figure 9, Figure 10). Corresponding sequences of *Myxococcus macrosporus* DSM14697 (for 16S rRNA, *gyrB*, *pgm*, *pyrG*, and *rpoB* gene sequence) and *Myxococcus fulvus* ATCC25199 (for 16S rRNA gene sequence) were provided from the Gene Bank/NCBI databases (Figure 10).

Thirteen myxobacterial isolates from three different sampling sites were evaluated using six housekeeping genes (*fusA*, *gyrB*, *lepA*, *pgm*, *pyrG* and *rpoB* genes) (Figure 9). All strains revealed at least two from six of mentioned housekeeping genes. However, the *fusA* gene could not be amplified for all isolates (Figure 9). *Myxococcus* spp. isolates, the most abundant strains within three different sampling sites, were selected for further analyses.

Table 14. Characteristics of five loci of *Myxococcus* spp. strains

Locus	Strain analyzed (n)	Nucleotides (%)				Frequency T/C/A/G (%)
		Conserved	Variable	Parsimony-informative	Total*	
16S rRNA	7	1214 (75.59)	318 (19.80)	293 (18.24)	1039/1606	20.2 26.2 23.3 30.3
gryB	6	1212 (96.11)	49 (3.89)	33 (2.61)	1207/1261	13.3 33.5 20.5 32.7
pyrG	6	192 (41.74)	222 (48.86)	36 (7.83)	415/460	18.0 33.4 16.1 32.6
rpoB	6	472 (53.70)	396 (45.05)	309 (35.15)	796/879	15.2 35.0 16.0 33.8
Pgm	5	961 (93.48)	67 (6.51)	31 (3.01)	1028/1028	12.5 36.3 16.7 34.5
Concatenated gyrB-pyrG-rpoB	6	1876 (72.15)	667 (25.65)	378 (14.54)	2417/2600	14.7 34.0 18.2 33.0
Concatenated gyrB-pyrG-rpoB-pgm	5	2910 (80.20)	656 (18.08)	386 (10.64)	3316/3638	14.2 34.5 17.7 33.6

* Mean number of nucleotides amplified, including gaps

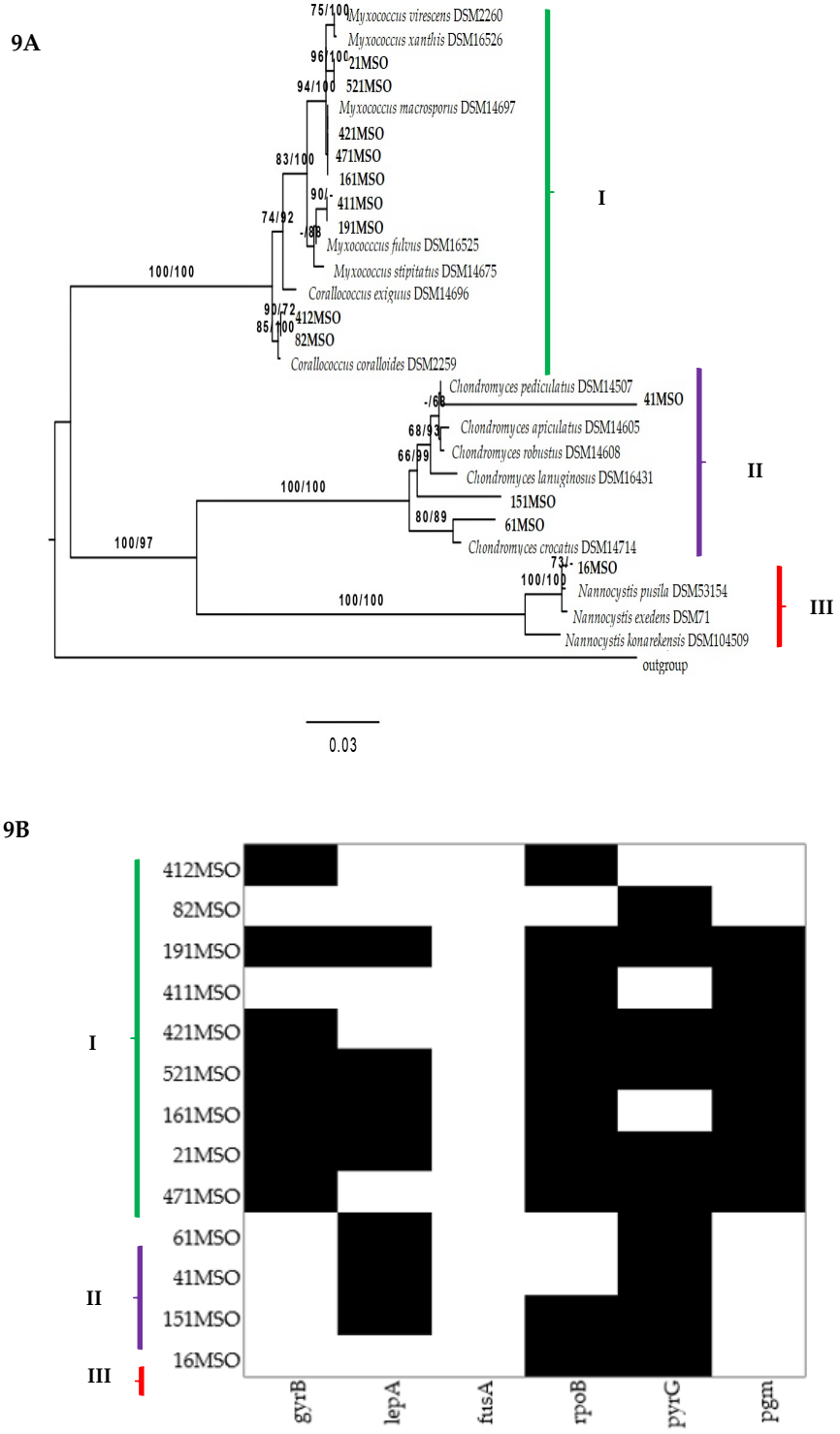


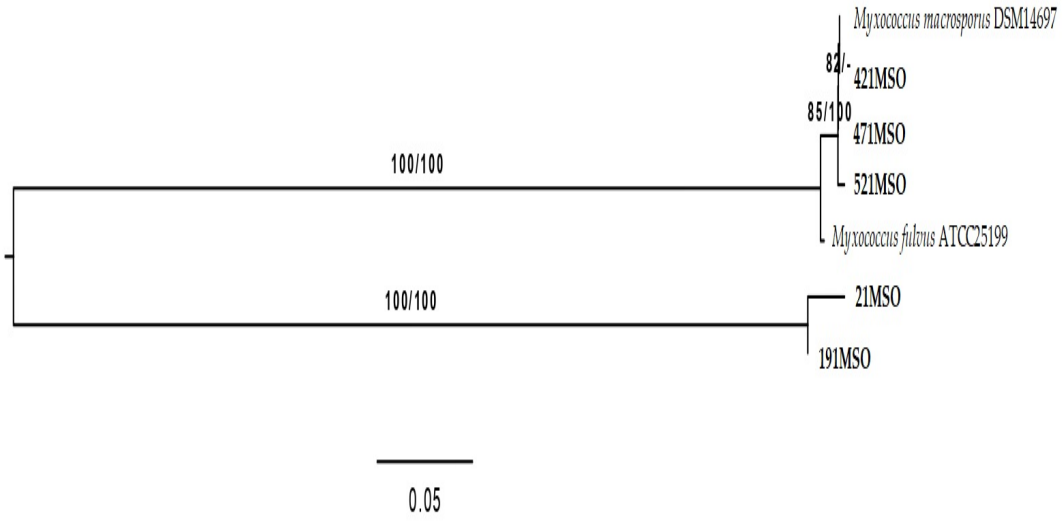
Figure 9. Phylogenetic tree 16S rRNA gene sequences of 15 strains based on 16S rRNA gene sequences (9A). The numbers above the branches are support values when larger than 60 % from Maximum likelihood/ML (left) and Maximum parsimony/MP (right) bootstrapping. Amplified sequence result of six housekeeping genes (9B). In heat map, housekeeping genes, unamplified genes (white color) and amplified genes (black color). Group I suborder *Cystobacterineae*, II suborder *Sorangiineae* and III suborder *Nannocystineae*.

As shown in Table 16, characteristics of five loci of myxobacterial isolates from mangroves were compared to the type strains of genus *Myxococcus* including strains 421MSO, 471MSO, 521MSO, 21MSO, 191MSO, *Myxococcus macrosporus* DSM14697 and *Myxococcus fulvus* ATCC25199. Multiple sequence alignments obtained 1606 bp for the 16S rRNA gene, 1261 bp for *gyrB* gene, 460 bp for *pyrG* gene, 1028 bp for pgm gene, 879 bp for rpoB gene, 2600 bp for the concatenated gryB-pyrG-rpoB genes, and 3628 bp for the concatenated gyrB-pyrG-rpoB-pgm genes. Furthermore, a highly conserved sequence has remained useful for reconstruction of deep phylogenetic relationships [81–83]. Therefore, these results clearly showed that concatenation of the housekeeping genes revealed higher resolution of distance matrix than 16S rRNA gene only.

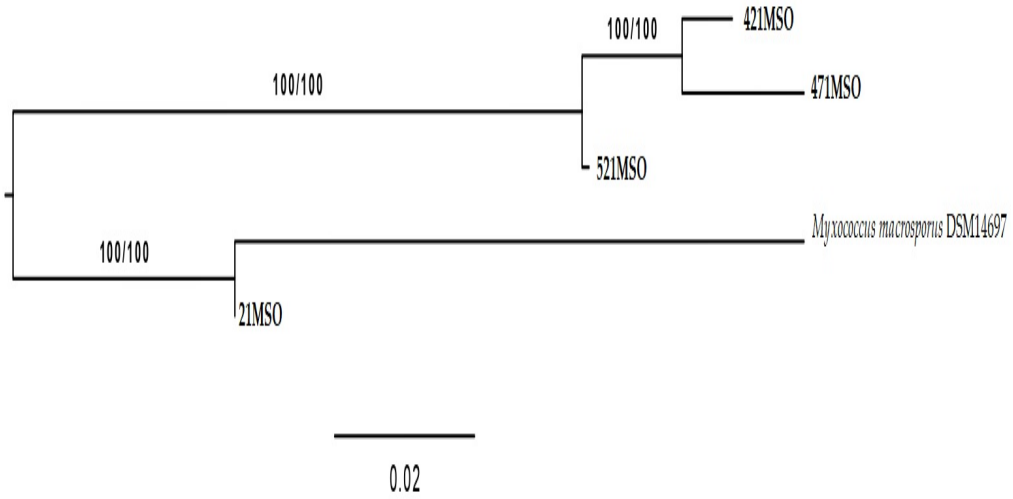
3.3.3. Phylogenetic tree analysis

The phylogenetic trees of 16S rRNA gene and concatenated housekeeping genes were drawn in Figure 10. The 16S rRNA gene revealed two clades including five myxobacterial isolates from mangroves and two type strains. First clade had two branches including the strains 421MSO, 471MSO, 521MSO that are closely related to *Myxococcus macrosporus* DSM14697 in the first branch. *Myxococcus fulvus* ATCC25199 separately in the second branch. Strain 21MSO, which is closely related to 191MSO, formed the second clade within the family *Myxococcaceae*.

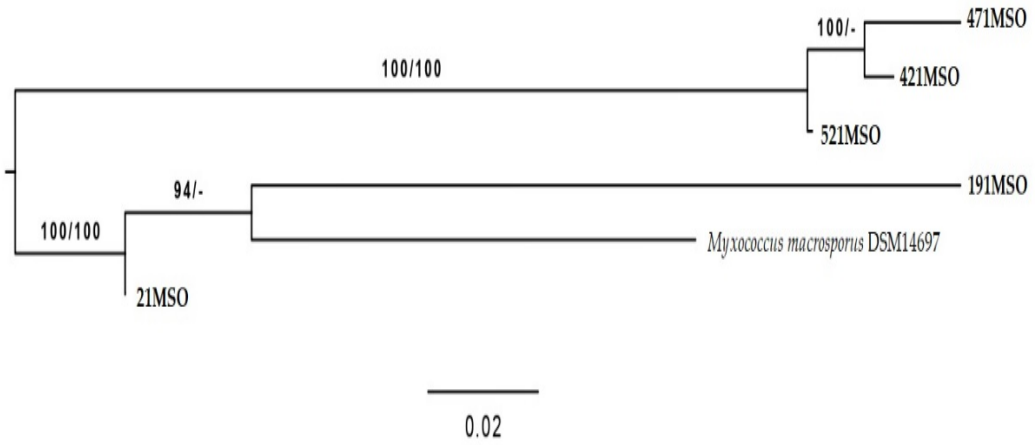
The concatenated gyrB-pyrG-rpoB-pgm genes and concatenated gryB-pyrG-rpoB genes highlighted a different phylogenetic tree from that of the 16S rRNA gene (Figure 10). *Myxococcus macrosporus* DSM14967 was shown to be shifted from the first clade to the second clade. Furthermore, strains 421MSO, 471MSO, and 521MSO were situated in different branches using both concatenated genes.



16S rRNA gene



Concatenated *gyrB-pyrG-rpoB-pgm* genes



Concatenated *gyrB-pyrG-rpoB* genes

Figure 10. Comparison of 16S rRNA gene-based and MLSA-based phylogenetic resolutions of Myxococcus spp. strains. Maximum likelihood (ML) tree inferred under the GTR+GAMMA model and rooted by midpoint rooting. The branches are scaled in terms of the expected number of substitutions per site. The numbers above the branches are support values when larger than 60 % from Maximum likelihood/ML (left) and Maximum parsimony/MP (right) bootstrapping.

3.3.4. Intraspecies and interspecies sequences similarities

In order to confirm the accuracy of the MLSA analysis, the pairwise distances matrix and sequences similarities were calculated by MEGA X software and Genbank/NCBI/BLAST database, respectively. The intraspecies and the interspecies sequences similarities of the individual gene and concatenated genes are sketched in Figure 11.

Similarity values of the intraspecies and the interspecies sequences overlapped almost in the individual gene except the *rpoB* gene. Strain 191MSO which belongs to the interspecies of *Myxococcus* sp. was not amplified with the *pgm* gene. In this study, the 16S rRNA gene gave similarity value approximately of 99 % and 98 % within and between species, respectively. In contrast to the 16S rRNA gene, the concatenated genes could be defined and distinguished within and between strains of the genus *Myxococcus*.

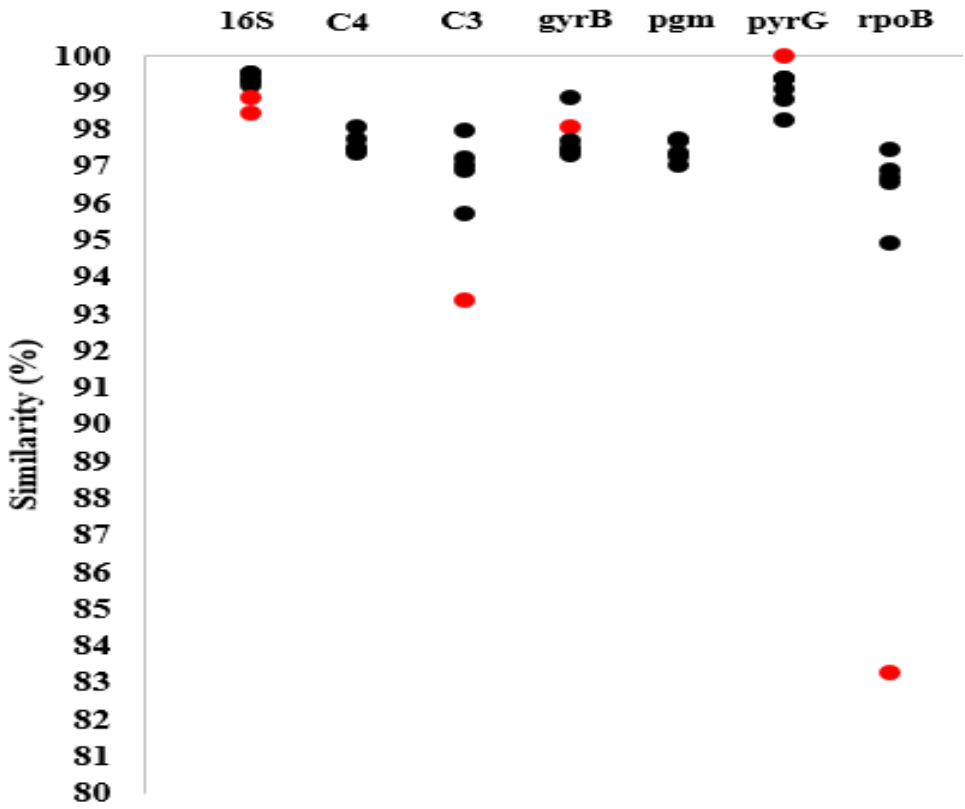


Figure 11. Taxonomic resolution based on ranges of intraspecies (black) and interspecies (red) similarity of *Myxococcus* spp. C4 = concatenated gryB-pyrG-rpoB-pgm genes; C3 = concatenated gyrB-pyrG-rpoB genes.

3.4. *Ohtaekwangia* sp. nov., gliding bacterium isolated from Indonesian mangroves and the related strains from HZI strain collection

3.4.1. Strain isolation

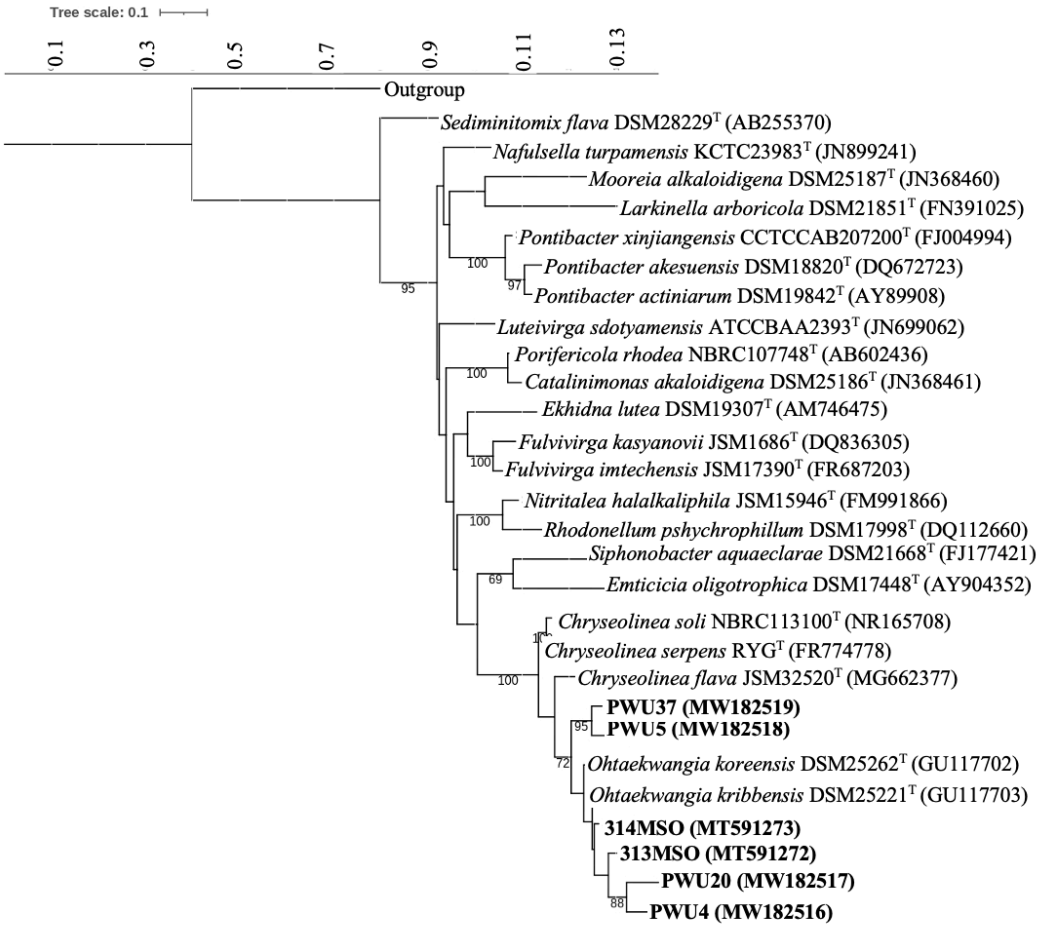


Figure 12. Phylogenetic tree 16S rRNA gene sequences of strain 313MSO, 314MSO and related strains (PWU) from HZI culture collection. The numbers above the branches are support values when larger than 60 % from Maximum Likelihood with the respective 1000 bootstrap values.

During this study, two strains 313MSO and 314MSO were isolated from Taman Muara Angke, Jakarta, and are closely related to *Ohtaekwangia kribbensis* with the similarity of 96.20 % and 99.12 %, respectively. We also used "*Pseudowuzel"*

(PWU) strains from the HZI culture collection to compare 16S rRNA gene sequences similarity of strain 313MSO and 314MSO.

The phylogenetic tree with 16S rRNA gene sequences of strain 313MSO, 314MSO and PWUs are mentioned in Figure 12. The levels of partial 16S rRNA gene sequence similarity between strain 313MSO, 314MSO and other bacterial taxa are acceptable to differentiate strains from the known genus *Ohtaekwangia* of the phylum *Bacteroidetes*. Strain 314MSO was more closed relative to the type strains of genus *Ohtaekwangia*, whereas strain 313MSO was more related to the PWU strains from the HZI culture collection. Therefore, we selected strain 313MSO and four PWU strains (see 3.4.4) for further deep characterization by a polyphasic approach. Type strains of the genus *Ohtaekwangia* were used as reference strains for comparison in this study.

3.4.2. Characteristics of strain 313MSO

Colonies of strain 313MSO were bright yellow and swarmed in radial pattern on VY/2 medium (Figure 13). The cells were rod shaped, 1-5 µm long, and proven to be Gram-negative on staining. The optimum growth was recorded at 30 °C with a pH range of 6.5-7.5, and without addition of NaCl while up to 1 % (w/v) of NaCl could still be tolerated.

A summary of morphological characteristics of strain 313MSO and the reference strains is presented in Table 15. Strain 313MSO exhibited neither catalase nor oxidase activity. Flexirubin type pigments could not be identified. It exhibited low-level resistance to gentamycin, but was sensitive to polymycin, kanamycin, ampicillin and chloramphenicol (not included in Table 15). The major polar lipids of strain 313MSO were phosphatidylinositol-mannosids, while the ones of the reference's, strain 3B-2 and 10AO were phosphatidylethanolamine. Strain 313MSO showed some differences of the morphological characteristics compared to the reference strains.

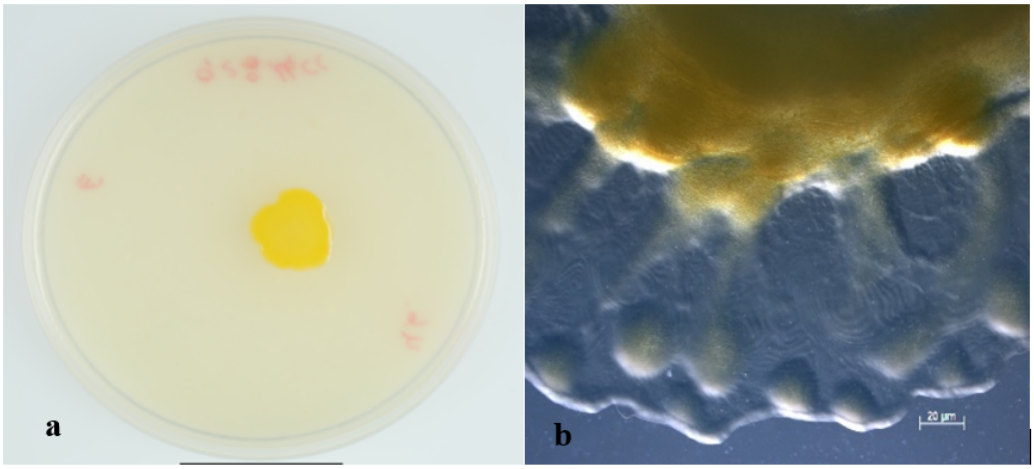


Figure 13**.** Morphological characteristics of strain 313MSO. (A) Growth of strain 313MSO on VY/2 agar medium. (B) Swarming of strain 313MSO on agar plate. Scale bars, 10-20 µm.

Table 15. Morphological Characteristics of Strain 313MSO.

*Note: + able; – unable; 3B2 = O. *koreensis* DSM25262^T; 10A0 = O. *kribbensis* DSM25221^T

Characteristic	**313MSO**	**3B2***	**10A0***
Cell Shape	Rod	Rod	Rod
Cell Size [µm]	1.0-5.0	1.0-5.0	1.5-7.5
NaCl Tolerances [w/v]			
0-1 %	+	+	+
2.5-10 %	-	-	-
Catalase	-	+	+
Oxidase	-	+	+
Flexirubin type pigment	-	+	+
Optimal growth	30 ºC	30 ºC	30 ºC
Optimal pH	6.5-7.5	6.5-7.5	6.5-7.5
Optimal medium	VY/2	R2A	R2A
Gram Staining	Negative	Negative	Negative
Antibiotic Resistance			
Gentamycin [50µg/mL]	+	-	+
Phospholipid	Phosphatidylinositol-mannosid	Phosphatidyl ethanolamine	Phosphatidyl ethanolamine

Enzyme activity analysis and assimilation of various substrates are shown in Table 18. Strain 313MSO could be clearly distinguished from its closely related type strains by enzyme activities and substrate assimilation. Strain 313MSO could assimilate acetoacetic acid, alpha-hydroxy-butyric acid, D-lactic acid methyl ester, D-serine, glycerol, L-alanine, L-lactic acid, methyl pyruvate, myo-inositol, N-acetyl neuraminic acid and sodium butyrate while references strains could not be able to assimilate all of them (Table 16). Moreover, strain 313MSO had no enzyme activities for alpha fucosidase, esterase and HIP-purate. However, the enzyme activities of strain 313MSO are similar to *Ohtaekwangia kribbiensis* 3B-2 based on APIZYM and APICAMPY test.

Table 16. Biochemical Properties of strain 313MSO and related Type Strains

*Note: + able; – unable; w = weak; 3B2 = O. *koreensis* DSM 25262^T; 10A0 = O. *kribbensis* DSM 25221^T

Characteristic	**313MSO**	**3B2***	**10A0***
Assimilation of (Biolog)			
3-Methyl Glucose	-	+	-
Acetic Acid	+	-	+
Acetoacetic Acid	+	-	-
Alpha-Hydroxy-Butyric Acid	+	-	-
Alpha-Keto-Glutaric Acid	-	-	+
Beta-Hydroxy-D, L-Butyric Acid	-	-	+
Beta-Methyl-D-Glucosidase	-	+	+
Bromo-Succinic Acid	-	-	+
Citric Acid	-	-	+
D-Aspartic Acid	-	+	+
D-Fructose-6-PO4	+	-	+
D-Galactose	+	-	+
D-Gluconic Acid	+	-	+
D-Lactic Acid Methyl Ester	+	-	-
D-malic Acid	-	-	+
D-Mannose	+	-	+
D-Saccharic Acid	-	-	+
D-Serine	+	-	-
Formic Acid	-	-	+
Gentiobiose	+	-	+
Glucuornicamide	+	+	+
Glucuronic Acid	-	-	+

Glycerol	+	-	-
Glycyl-L-Proline	-	+	+
Guanidine HCl	-	-	+
L-Alanine	+	-	-
L-Arginine	-	-	+
L-Fucose	+	+	+
L-Galactonic Acid Lactone D-Gluconic Acid	-	+	+
L-Glutamic Acid	+	-	+
Linomycin	+	-	+
Lithium Chloride	+	-	+
L-Lactic Acid	+	-	-
L-Malic Acid	-	-	+
L-Rhamnose	+	-	+
L-Serine	+	-	+
Methyl Pyruvate	+	-	-
Minocycline	+	-	+
Mucic Acid	-	-	+
myo-Inositol	+	-	-
N-Acetyl Neuraminic Acid	+	-	-
N-Acetyl-D-Galactosamine	+	-	+
Nalidixic Acid	+	-	+
p-Hydroxy-Phenylacetic Acid	-	-	+
Potassium Tellurite	+	-	+
Propionic Acid	-	-	+
Quinic Acid	-	+	+
Sodium Butyrate	+	-	-
Sucrose	+	-	+
Tetrazolium Violet	-	-	+
Tetrazolum Blue	+	-	+
Troleandomycin	+	-	+
Vancomycin	+	-	+
APIZYM			
Trypsin	W	+	-
Naphtol-AS-B1-Phosphohydrolase	+	+	-
Beta glucosidase	+	+	-
Alpha fucosidase	-	+	-
API CAMPY			
Esterase	-	+	-
HIP-purate	-	+	-
Gamma Glutamyl Transferase	+	-	-

The fatty acid profiles of strain 313MSO and of related type strains of the genus *Ohtaekwangia* are shown in Table 17. The existence of $C_{18:0}$ and the lower proportion of Iso $C_{16:0}$ in strain 313MSO differed from the related type strains. Overall, strain 313MSO has different characters of the biochemical properties to its related type strains.

Table 17. Fatty Acid Profiles of Strain 313MSO and related of Type Strains

*Note: + able; – unable; w = weak; 3B2 = O. *koreensis* DSM25262^T; 10A0 = O. *kribbensis* DSM25221^T

Fatty Acid	313MSO	3B2*	10A0*
Straight-chain			
$c_{15:0}$	-	-	2,1
$c_{16:0}$	-	-	22,2
$c_{18:0}$	1,59	-	-
Branched			
Iso $c_{14:0}$	-	-	1,7
Iso $c_{15:0}$	-	20,4	30,2
Iso $c_{16:0}$	3,8	9,5	4
Iso $c_{17:0}$	-	-	7,4
Unsaturated			
$c_{16:1}$ ω7c	-	55,2	27,4
Unknown			
ECL 11.864	-	6,2	3,3
ECL 22.207	-	8,7	1,7

3.4.3. Antimicrobial activity

Twenty-three crude extracts were examined for antimicrobial activity against pathogenic microbes including five Gram-negative, two Gram-positive bacteria and three fungi (Figure 14). The antimicrobial assay (MIC) showed that most of the crude extracts exhibited growth inhibition against *Staphylococcus aureus* while the highest activities were obtained in Pol and 1/10 CY/H media with a MIC value of 33.33 µg/mL. Moreover, the crude extracts in 1/10 E and MA media showed

inhibition of *Escherichia coli* and *Pseudomonas aeruginosa* with a MIC value of 66.67 µg/mL.

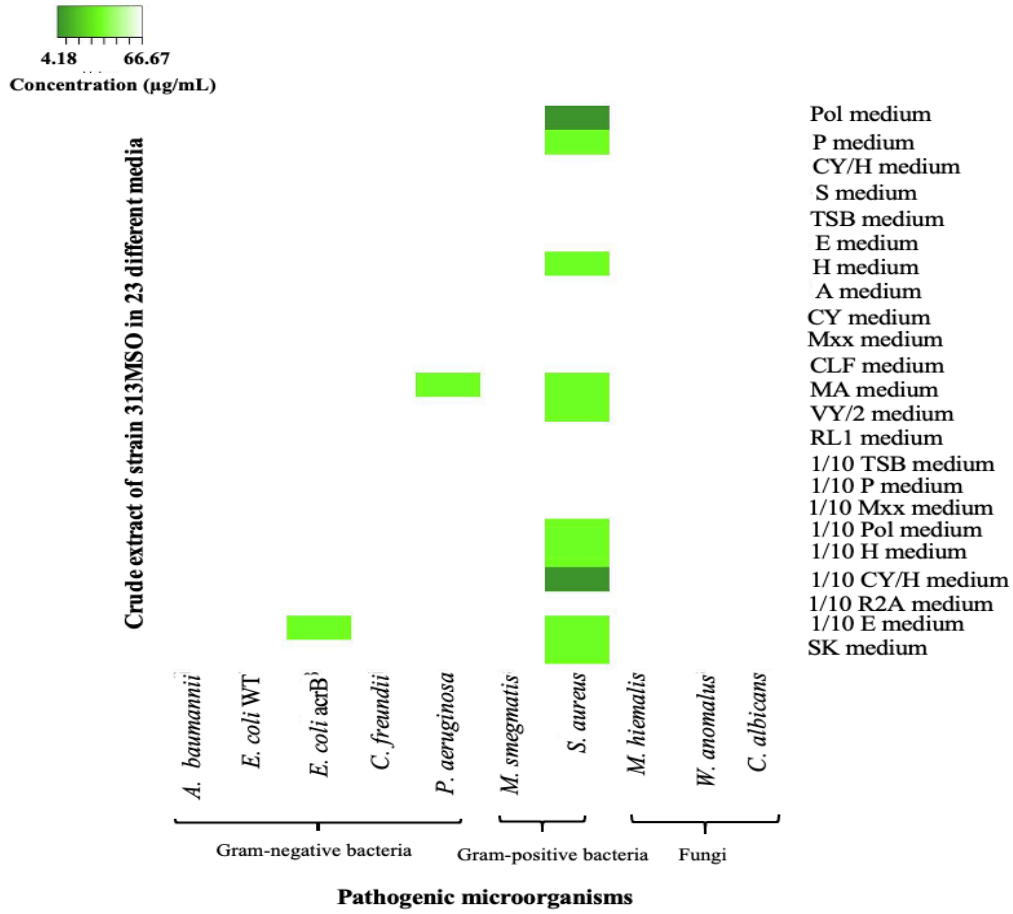


Figure 14**.** The antimicrobial activity of crude extracts from 23 different media tested (Y-axis) acting against 10 different microbial pathogens (X-axis). The different colors indicate different minimum inhibitory concentrations (MIC) of crude extracts exerting inhibitory effects. MIC values were calculated by serial dilution with the highest concentration of 66.7 µg/mL (weak antimicrobial activity) diluted 8 times to the lowest concentration of 0.52 µg/mL (strong antimicrobial activity).

Further, Pol and 1/10 CY/H media with a MIC value of 33.33 µg/mL were selected and analyzed by HPLC-UV-HRESIMS. An active fraction from these media was identified as fatty acid group based on in-house database myxobase (Figure S4). However, some non-active fractions in Pol medium, were detected as marinoquinolines A and its five derivates (B-F) (Figure S4) which were identified in previous study by Okanya *et al*. [22].

Overall, antimicrobial activities were observed against 30 % (3 of 10) of pathogenic microorganisms and 14 myxobacterial crude extracts were not active against any of those microorganisms. Antimicrobial activity against *Candida albicans* DSM1665, *Acinetobacter baumannii* DSM30008, *Escherichia coli* WT BW25113, *Citrobacter freundii* DSM30039, *Mycobacterium smegmatis* ATCC700084, *Mucor himalis* DSM2656 and *Wickerhamomyces anomalus* DSM6766 was not observed for the crude extracts produced from the gliding bacterium.

3.4.4. Related Strains from HZI Culture Collection

3.4.4.1. Isolation and Ecology

Strains PWU4^T, PWU5^T, PWU20^T and PWU37^T were obtained from the microbial culture collection group at Helmholtz Center for Infection Research (HZI) Germany. Strain PWU4^T, PWU20^T and PWU37^T were revealed from soil samples collected in May 1990 at Braunschweig, Germany (52.22090 N 10.50902 E), in May 1989 at Lucknow, Uttar Pradesh, India (26.8684 N 80.90979 E) and in September 1991 at Braunschweig (52.21501 N 10.53329 E), respectively. Strain PWU5^T was revealed from a face of sheep with plant residues collected in July 1988 at Crete Island (35.2463 N 25.09705 E).

The four strains were isolated using a dilution method on agar plates following the protocol of Reichenbach [16], maintained in E medium and kept directly with this medium at -80^{o}C for long-term preservation. For physiological and chemotaxonomy test, the four strains were grown without NaCl in E broth medium at their optimum pH and temperatures including pH 7 and 30^{o}C for strain PWU4^T, pH 7 and 28^{o}C for strain PWU20^T and strain PWU5^T, pH 7.4-8.0 and 34^{o}C for strain PWU37^T. *Ohtaekwangia koreensis* 3B2 DSM 25262^T and *Ohtaekwangia kribbensis* 10AO DSM 25221^T were used as references strains and were grown under the same culture conditions.

3.4.4.2. Characteristics of genome

The phylogenetic tree of 16S rRNA gene showed the affiliations of strain PWU4^T, PWU5^T, PWU20^T and PWU37^T belonging to the family *Cytophagaceae* but also showed that there are no close relatives described (Figure 1) and they shared in range 91.3 up to 97.5 % 16S rRNA gene sequence similarity within each other. On the report of the NCBI database, the closest relatives of strain PWU4^T were *Ohtaekwangia koreensis* DSM 25262^T (92.1 % 16S rRNA gene sequence similarity), *Ohtaekwangia kribbensis* DSM 25221^T (92.0 %) and *Chryseolinea soli* KIS68-18^T (91.0 %), whereas the closest relatives of strain PWU5^T were *Ohtaekwangia koreensis* DSM 25262^T (93.6 % 16S rRNA gene sequence similarity), *Ohtaekwangia kribbensis* DSM 25221^T (93.1 %) and *Chryseolinea serpens* RYG^T (92.3 %). The closest relatives of strain PWU20^T were *Ohtaekwangia kribbensis* DSM 25221^T (92.5 % 16S rRNA gene sequence similarity), *Ohtaekwangia koreensis* DSM 25262^T (92.0 %) and *Chryseolinea soli* KIS68-18^T (90.6 %). Moreover, the closest relatives of strain PWU37^T were *Ohtaekwangia kribbensis* DSM 25221^T (93.7 % 16S rRNA gene sequence similarity), *Ohtaekwangia koreensis* DSM 25262^T (93.2 %) and *Chryseolinea soli* KIS68-18^T (92.0 %).

Draft genome sequences of four strains were described previously [84]. The DNA G+C content of strain PWU4^T, PWU5^T, PWU20 ^{T} and PWU37^T were 50.2 mol %, 51.6 mol %, 39.8 mol % and 53.8 mol %, respectively. Further, the differences in G+C content were more than 2.3 % and thus supporting distinct species [85]. The pairwise digital DNA-DNA hybridization (dDDH) revealed a value of 13 % to 50 % and confirmed that all of the strains represent novel species.

The average nucleotide identity (ANI) values between the genome of the strains and their closest relatives were calculated with the OrthoANIu algorithm using the EZ-Genome web service [86]. Strain PWU4^T; PWU5^T; PWU20 ^{T}; and PWU37^T shared ANI values of 69.2 %; 69.9 %; 69.5 % and 69.7 % with *Ohtaekwangia koreensis* DSM25262^T and values of 68.2 %; 67.6 %; 68.7 % and 67.6 % with *Chryseotalea sanaruensis* Ys^T, respectively. The low ANI value below the

threshold 95.0 %-96.0 % [87] confirms that all of strains represent different species for each other.

3.4.4.3. Characteristic of physiology and chemotaxonomy

Phenotypic characterization was performed following to the protocol previously described [88–90]. Morphological characteristic of the strain was observed using a light microscope (Zeiss Axio Scope A1. Microscope) with Axio-Vision Rel. 4.8 software. The cells of strain PWU4^T, PWU5^T, PWU20^T and PWU37^T were straight rods, 2.32-7.62 µm in length, stained Gram-negative and form yellow colonies on E medium.

Growth at various temperature, pH and NaCl concentrations were carried out aerobically on E agar medium. To determine the optimal temperature and pH, duplicate plates were incubated at 4-44^{o}C and also at pH 5.0-9.5 as described previously [84]. Strains PWU4^T and PWU20^T grew at 21-40^{o}C (optimum at 28-30^{o}C), while strains PWU5^T and PWU37^T grew at 21-34^{o}C (optimum at 28-34^{o}C). Moreover, strain PWU4^T growth at pH 5.5-8.0 (optimum at pH 7), strain PWU5^T growth at pH 6.5-8.5 (optimum at pH 7), strain PWU20^T growth at pH 6.5-9.0 (optimum at pH 7) while strain PWU37^T growth at pH 5.0-9.5 (optimum at pH 7.4-8.0). In contrast to previous study, the reference strains [88] optimally grew at 30^{o}C in a range of 10-39^{o}C and pH 6.5-7.5 (Table 20). Salt tolerance was performed in a range of 0.2-1.6 % NaCl (w/v). Strain PWU4^T, PWU5^T, PWU20^T and PWU37^T could tolerate a concentration up to 0.4, 0.6, 0.8 and 1.0 % NaCl (w/v), respectively. However, in this study the reference strains such as members of the genus *Ohtaekwangia,* which were isolated from marine environment, tolerated a concentration up to 0.2 % NaCl (w/v). Other of the closest genera such as *Chryseotaela* tolerated up to 1.0 % of NaCl (w/v) [90] and *Chryseolinea* up to 0.1 % of NaCl (w/v) [89]. Anaerobic growth was performed using E agar plates with Anaerocult P (Merck) in a candle jar [91] among 3 weeks of incubation. No growth was observed for all the strains under anaerobic conditions. Reichenbach [16] mentioned that a few of the member of *Cytophagaceae* grow microaerophilic, capnophilic (CO_2-requiring) or facultative anaerobic.

Catalase and oxidase activities were performed following Yoon *et al*. [90] and the production of flexirubin-type pigments was tested following Reichenbach [16]. Catalase and oxidase activities along with flexirubin-type pigments for all the strain were negative, also, for the reference strains (Table 18).

Carbon sources utilization assay was carried out in duplicate using E broth medium along with the Gen III MicroPlate system (Biolog) following to the manufacturer's protocol. Strain PWU4^T, PWU5 ^{T}, PWU20 ^{T} and PWU37 ^{T} were able to metabolize D-galactose and glutamic acid and are not able to use dextrin and N-acetyl-D-glucosamine (Table 18).

Table 18. Major phenotypic distinguishing strain PWU4^T, PWU5^T, PWU20^T and PWU37^T with closest genera.

*Strain: 1, PWU4^T; 2, PWU5^T; 3, PWU20^T; 4, PWU37^T; 5, *Ohtaekwangia koreensis* 3B2 DSM25262^T and 6, *Ohtaekwangia kribbensis* 10AO DSM25221^T. +, positive; w. weakly activities, - negative, **taken from Yoon et al. [75]

Characteristic*	**1**	**2**	**3**	**4**	**5**	**6**
Cell morphology	Rod	Rod	Rod	Rod	Rod	Rod
Cell length [µm]	2.56-6.67	4.37-7.62	2.32-6.41	3.1-6.43	1.0-5.0	1.5-7.5
Temperature range of growth [°C]	21-40	21-34	21-40	21-34	10-39	10-39
Optimal temperature [°C]	30	28	28	34	30	30
pH range of growth	5.5-8.0	6.5-8.5	6.5-9.0	5.0-9.5	5.5-9.0	4.5-9.0
Optimal pH	7	7	7	7.4-8.0	6.5-7.5	6.5-7.5
NaCl tolerance [%NaCl, w/v]	0-0.4	0-0.6	0-0.8	0-1.0	0-0.2	0-0.2
Flexirubin type pigment	-	-	-	-	+	+
Catalase	-	-	-	-	+	+
Oxidase	-	-	-	-	+	+
Enzyme activity (Api®ZYM, Api®CAMPI) :						
Esterase (C4)	+	w	W	w	-	-
Esterase lipase (C8)	+	w	W	w	-	-

Lipase (C14)	w	w	w	W	-	-
Valine arylamidase	+	+	+	+	+	-
Cystine arylamidase	+	+	+	+	-	-
Trypsin	+	-	w	-	+	-
Chymotrypsin	+	-	+	-	-	-
Phosphatase acid	+	+	+	+	+	-
Naphthol-AS-B1-Phosphohydrolase	+	+	+	+	+	-
α-galactosidase	+	+	+	+	+	-
β-galactosidase	+	+	+	+	+	-
β-glucoronidase	w	-	+	-	-	-
α-glucosidase	+	+	+	+	+	-
β-glucosidase	+	+	+	+	+	-
α-mannosidase	+	-	+	-	+	-
α-fucosidase	+	-	-	+	+	-
Urease	+	-	+	-	-	-
Hippurate	+	-	+	-	+	+
γ-glutamyl transferase	+	+	+	+	-	-
Reduction of tetrazolium	+	+	+	+	-	-
Antibiotic resistance:						
Gentamycin [50 µg/ml]	+	+	-	+	-	+
G+C contents [mol%]	50,2	51,6	39,8	53,8	42,8**	44,6**
Carbon sources utilization:						
D-Cellobiose	-	+	-	+	+	+
L-Fucose	-	+	-	+	+	+
D-Arabitol	-	+	-	+	-	-
D-Gluconic Acid	+	-	+	-	+	+
Methyl Pyruvate	+	-	+	-	-	-
L-Malic Acid	-	+	-	+	-	+
α-Keto-Butyric-Acid	-	+	-	+	-	-

Enzyme activities were performed using the API ZYM [76] and API CAMPY [77] system (bioMérieux), according to the protocols of manufacturer. Strain PWU4^{T}, PWU5 ^{T}, PWU20 ^{T} and PWU37 ^{T} along with reference strains (Table 18) were positive for phosphatase alkaline, leucine arylamidase, N-Acetyl-β-glucosamidase,

L-arginine arylamidase, L-aspartame arylamidase and alkaline phosphatase. Negative test was obtained for pyrrolidinyl arylamidase, reduce nitrate and H_2S production. Antibiotic resistances was tested on E agar medium following disc-diffusion plate method [78]. Strain PWU4^T, PWU5 ^{T}, PWU20 ^{T} and PWU37 ^{T} were resistant to Polymycin [50µg/mL], Kanamycin [50µg/mL], Ampicillin [100 µg/mL] and vice versa to Chloramphenicol [30µg/mL].

Strain PWU4^T, PWU5 ^{T}, PWU20 ^{T} and PWU37 ^{T} were cultured on M broth medium. After seven days, during the late logarithmic phase, the freeze-dried cells were prepared for detection of the major isoprenoid quinone, the polar lipid and the cellular fatty acid of strains. In brief, 200 ml well-grown cultures were centrifuged at 9,000 rpm for 10 min and the pellet was washed three times following centrifugation at 9,000 rpm for 10 min and continued by freeze-drying for two days. The isoprenoid quinone was tested following Komagata and Suzuki [92] and analyzed further using HPLC (Agilent 1260 Series; Agilent technology USA). The polar lipid was determined by two-dimensional TLC [79, 93].

Table 19. Cellular fatty acid composition of strain PWU4^T, PWU5^T, PWU20^T and PWU37^T with closest genera.

*Strain: 1, PWU4^T; 2, PWU5^T; 3, PWU20^T; 4, PWU37^T; 5, *Ohtaekwangia koreensis* 3B2 DSM25262^T and 6, *Ohtaekwangia kribbensis* 10AO DSM25221^T. +, positive; - negative. Values are percentages of total fatty acids. -, not detected, ECL, equivalent chain-length.

Characteristic*	**1**	**2**	**3**	**4**	**5**	**6**
Straight-chain						
$c_{14:0}$	1,9	-	1,3	1,0	-	-
$c_{15:0}$	1,8	2,3	-	1,6	-	2,1
$c_{16:0}$	-	2,8	9,7	9,2	-	22,2
$c_{17:0}$	-	1,3	-	-	-	-
$c_{18:0}$	2,7	-	1,1	-	-	-
Branched						
iso $c_{13:0}$	-	-	-	-	-	-
iso $c_{14:0}$	-	-	-	-	-	1,7
iso $c_{15:0}$	22,0	26,9	43,6	38,2	20,4	30,2
iso $c_{16:0}$	12,9	2,2	1,4	-	9,5	4,0
iso $c_{17:0}$	-	-	1,2	1,0	-	7,4

Unsaturated						
$c_{15:1}$ ω7c	1,4	2,8	0,8	-	-	-
$c_{16:1}$ ω5c	-	43,8	-	38,5	-	-
$c_{16:1}$ ω7c	32,5	16,6	32,0	9,0	55,2	27,4
$c_{16:1}$ ω8c	-	-	-	0,7	-	-
$c_{17:1}$ ω7c	-	-	1,0	-	-	-
$c_{18:1}$ ω9c	8,9	-	3,4	-	-	-
$c_{18:2}$ ω6,9c	13,6	-	4,5	-	-	-
Hydroxy						
$C_{14:0}$ 2-OH	1,0	-	-	-	-	-
$C_{16:0}$ 2-OH	1,3	-	-	-	-	-
Unknown						
ECL 11.864	-	-	-	-	6,2	3,3
ECL 12.558	-	1,2	-	-	-	-
ECL 16.089	-	-	-	0,7	-	-
ECL 22.207	-	-	-	-	8,7	1,7

Furthermore, the Sherlock Microbial Identification System (MIDI) was used for identifying cellular fatty acids [94]. The major respiratory quinone is menaquinone MK-7 for all the strain. The menaquinone MK-7 was also found in closest genera (Table 18) and almost the member of the phylum *Bacteroidetes* [75, 89, 90] except the family *Flavobacteriaceae*, which have menaquinones of type 6 (MK6) [95]. The major polar lipids of strain PWU4^T, PWU5^T, PWU20^T and PWU37^T were phosphatidylethanolamine (PE) and another unknown polar lipid. The results of fatty acids were shown in Table 19, along those reference strains obtained in this study. Saturated and monounsaturated fatty acids with iso $c_{15:0}$ and $c_{16:1}$ ω7c were performed in all the strain including reference strains. The major fatty acid of strain PWU4^T, PWU5^T, PWU20^T and PWU37^T were $c_{16:1}$ ω7c (32.5 %), $c_{16:1}$ ω5c (43.8 %), iso $c_{15:0}$ (43.6 %) and $c_{16:1}$ ω5c (38.5 %), respectively. In contrast to strain PWU4^T where $c_{16:1}$ ω7c was found to be the major fatty acid but was much less abundant in strain PWU37^T (9.0 %). Moreover, $c_{16:1}$ ω5c as the major fatty acids in strain PWU5^{T}and PWU37^T but vice versa to strain PWU4^T and PWU20^T. McBride *et al*. [95] highlighted that branched, unsaturated or hydroxyl fatty acids represent the predominant cellular fatty acids in most genera.

Morphological, biochemical, physiological and phylogenetically characteristic of strains PWU4^T, PWU5 ^{T}, PWU20 ^{T} and PWU37 ^{T} confirmed their status within the

family *Cytophagaceae.* However, obtained genetic data between strains PWU4^{T}, PWU5^{T}, PWU20^{T}, PWU37^{T} and related genera were more important to differentiate them from known genera of the family *Cytophagaceae.* Therefore, strains PWU4^{T}, PWU5^{T}, PWU20^{T} and PWU37^{T} should be classified in two new genera within the family *Cytophagaceae.* Furthermore, a phylogenetic tree based on genomic of all the strain revealed strain PWU4^{T} and PWU20 ^{T} could be together in one genus with different species (the low ANI value 69.73 %) and other strain PWU37 ^{T} and PWU5 ^{T} also could be together in one genus with different species (the low ANI value 83.85 %)

3.4.4.4. Protologue

3.4.4.4.1. **Description of *Cryseosolum* gen. nov.**

Cryseosolum (Cry.se.o.so`lum. Gr. adj. *chryseos*, golden; *solum*, soil; N.L. *Cryseosolum* s yellow-colored bacteria from soil).

Gram-stain-negative, non-sporulating, non-motile, mesophilic, heterophilic and aerobic bacteria. Cells are rod-shaped and catalase- as well as oxidase-negative. Growth observed on D-gluconic acid and methyl pyruvate. The major cellular fatty acids are *iso*-$C_{15:0}$ and $C_{16:1}$ w7c. The major respiratory quinone is menaquinone-7 (MK-7). The major identified polar lipids were phosphatidylethanolamine (PE). Of the class *Cytophagia*, order *Cytophagales*, phylum *Bacteriodetes.* The type species is *Cryseosolum histidinii.*

3.4.4.4.1.1.**Description of *Cryseosolum histidinii* sp. nov.**

Cryseosolum histidinii (his.ti.din`ii. N.L. mask. adj. *histidinii* pertaining to histidine, an amino acid, which can be utilized by this strain).

In addition to the characteristic listed in the genus description, the species has the following characteristics: Cells are 2.56-6.67 µm in long and appear as single cells. Colonies are irregular and yellow on E agar plate after 5 days of cultivation. Growth occur between 21 and 40 ºC (optimum 30 ºC), between pH 5.5 and 8.0 (optimum pH 7) and tolerance with occur below 0.4 % (w/v) of NaCl. Positive for phosphatase alkaline, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, phosphatase acid, naphthol-AS-B1-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosamidase, α-mannosidase, α-fucosidase, urease, esterase and alcaline phosphatase. Weakly activities on lipase (C14) and β-glucoronidase. No growth is observed on dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, α-D-lactose, D-melibiose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, α-

D-glucose, D-mannose, L-fucose, D-arabitol, glycerol, gelatin, glycyl-L-proline, L-alanine, L-arginine, L-serine, pectin, D-galacturonic acid, L-galactonic acid lactone D-gluconic acid, glucuronic acid, glucuornicamide, mucic acid, D-lactic acid methyl ester, citric acid, D-malic acid, L-malic acid, bromo-succinic acid, tween 40, γ-amino-butryric acid, α-keto-butyric-acid, acetoaacetic acid, and acetic acid.

The type strain is PWU4^{T} (=DSM 111594^{T} = NCCB 100798^{T}) isolated from a soil sample collected in May 1990 at Braunschweig, Germany (52.22090 N 10.50902 E). The DNA G+C content of the type strain is 50,2 mol %. The 16S rRNA gene and whole-genome sequences have been deposited in GenBank/EMBL/DDBJ under accession numbers MW182516 and ERR4837147, respectively.

3.4.4.4.1.2.**Description of *Cryseosolum indiensis* sp. nov.**

Cryseosolum indiensis (in.di.en. `sis. N.L. fem. adj. *indiensis* referring to India from which soil was isolated).

In addition to the characteristic listed in the genus description, the species has the following characteristics: Cells are 2.32-6.41 μm long and appear as single cells. Colonies are irregular and yellow on E agar plate after 5 days of cultivation. Growth occur between 21 and 40 ºC (optimum 28 ºC), between pH 6.5 and 9.0 (optimum pH 7) and tolerance with occur below 0.8 % (w/v) of NaCl. Positive for phosphatase alkaline, leucine arylamidase, valine arylamidase, cystine arylamidase, chymotrypsin, phosphatase acid, naphthol-AS-B1-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucoronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosamidase, α-mannosidase, urease, esterase and alcaline phosphatase. Weakly activities on esterase (C4), esterase lipase (C8), lipase (C14), trypsin and no activities for α-fucosidase. No growth is observed on dextrin, D-maltose, D-cellobiose, gentiobiose, N-acetyl-D-glucosamine, N-acetyl-β-D-galactosamine, D-fructose, D-fucose, L-rhamnose, inosine, D-sorbtiol, D-mannitol, D-arabitol, myo-inositol, D-fructose-6-PO4, L-alanine, L-arginine, L-histidine, L-galactonic acid

lactone D-gluconic acid, p-hydroxy-phenylacetic acid, L-malic acid, bromo-succinic acid, tween 40, α-keto-butyric-acid, propionic acid, and acetic acid.

The type strain is PWU20^T (=DSM 111595^T = NCCB 100800^T) isolated from a soil samples collected in May 1989 at Lucknow, Uttar Pradesh, India (26.8684 N 80.90979 E). The DNA G+C content of the type strain is 39.8 mol %. The 16S rRNA gene and whole-genome sequences have been deposited in GenBank/EMBL/DDBJ under accession numbers MW182517 and ERR4837148, respectively.

3.4.4.4.2. **Description of *Reichenbachia* gen. nov.**

Reichenbachia (rei.chen.bach`i.a. N.L. fem. Adj. *reichenbachia* referring to Hans Reichenbach, who was a famous Germany microbiologist in research on gliding bacteria).

Gram-stain-negative, non-sporulating, non-motile, mesophilic, heterophilic and aerobic bacteria. Cells are rod-shaped and catalase- as well as oxidase-negative. Growth observed on D-cellobiose, L-fucose, D-arabitol, L-malic acid, and α-keto-butyric-acid. The major cellular fatty acids are *iso*-$C_{15:0}$ and $C_{16:1}$ w5c. The major respiratory quinone is menaquinone-7 (MK-7). The major identified polar lipids were phosphatidylethanolamine (PE). Of the class *Cytophagia*, order *Cytophagales*, phylum *Bacteriodetes.* The type species is *Reichenbachia cretensis.*

3.4.4.4.2.1.Description of *Reichenbachia cretensis* sp. nov.

Reichenbachia cretensis (cre.ten.`sis. N.L. fem. adj. *cretensis* referring to Crete, from which soil was isolated).

In addition to the characteristic listed in the genus description, the species has the following characteristics: Cells are 4.37-7.62 µm long and appear as single cells. Colonies are irregular and yellow on E agar plate after 5 days of cultivation. Growth occur between 21 and 34 ºC (optimum 28 ºC), between pH 6.5 and 8.5 (optimum

pH 7) and tolerance with occur below 0.6 % (w/v) of NaCl. Positive for phosphatase alkaline, leucine arylamidase, valine arylamidase, cystine arylamidase, phosphatase acid, naphthol-AS-B1-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosamidase, esterase and alcaline phosphatase. Weakly activities on esterase (C4), esterase lipase (C8), lipase (C14) and no activities for trypsin, chymotrypsin, β-glucoronidase, α-mannosidase, α-fucosidase and urease. No growth is observed on dextrin, D-turanose, α-D-lactose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, L-arginine, L-histidine, L-pyroglutamic acid, D-gluconic Acid, glucuronic acid, glucuornicamide, mucic acid, quinic acid, D-saccharic acid, methyl pyruvate, citric acid, γ-amino-butryric acid, and β-hydroxy-D,L-butyric acid.

The type strain is PWU5^{T} (=DSM 111596^{T} = NCCB 100799^{T}) isolated from faces sheep with plant residues collected in July 1988 at Crete Island (35.2463 N 25.09705 E). The DNA G+C content of the type strain is 51.6 mol %. The 16S rRNA gene and whole-genome sequences have been deposited in GenBank/EMBL/DDBJ under accession numbers MW182518 and ERR4837149, respectively.

3.4.4.4.2.2.Description of *Reichenbachia soli* sp. nov.

Reichenbachia soli (so.li. N.L. mask. Adj. soli referring to soil, where it was isolated from Braunschweig, Germany).

In addition to the characteristic listed in the genus description, the species has the following characteristics: Cell are 3.1-6.43 μm long and appear as single cells. Colonies are irregular and yellow on E agar plate after 5 days of cultivation. Growth occur between 21 and 34 ^{o}C (optimum 34 ^{o}C), between pH 5.0 and 9.5 (optimum pH 7.4 until 8.0) and tolerance with occur below 1.0 % (w/v) of NaCl. Positive for activities phosphatase alkaline, leucine arylamidase, valine arylamidase, cystine arylamidase, phosphatase acid, naphthol-AS-B1-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosamidase, α-fucosidase, esterase and alcaline phosphatase. Weakly activities

for of esterase (C4), esterase lipase (C8), lipase (C14) and no activities for trypsin, chymotrypsin, β-glucoronidase, α-mannosidase, and urease. No growth is observed on dextrin, D-maltose, gentiobiose, D-turanose, D-raffinose, α-D-lactose, D-melibiose, D-salicin, N-acetyl-D-glucosamine, N-acetyl-β-D-galactosamine, N-acetyl-D-galactosamine, α-D-glucose, D-frucose, D-sorbtiol, myo-inositol, D-glucose-6-PO4, D-fructose-6-PO4, D-aspartic acid, D-serine, gelatin, L-alanine, L-arginine, L-aspartic Acid, L-histidine, pectin, L-galactonic acid lactone D-gluconic acid, D-gluconic acid, glucuornicamide, mucic acid, p-hydroxy-phenylacetic acid, methyl pyruvate, D-lactic acid methyl ester, L-lactic acid, citric acid, α-keto-glutaric acid, D-malic acid, bromo-succinic acid, tween 40, α-hyydroxy-butyric acid, β-hydroxy-D,L-butyric acid, acetoaacetic acid, propionic acid, acetic acid and formic acid.

The type strain is PWU37^{T} (=DSM 111597^{T} = NCCB 100801^{T}) isolated from a soil samples collected in September 1991 at Braunschweig, Germany (52.21501 N 10.53329 E). The DNA G+C content of the type strain is 53.8 mol %. The 16S rRNA gene and whole-genome sequences have been deposited in GenBank/EMBL/DDBJ under accession numbers MW182519 and ERR4837150, respectively.

The strain will be described in the International Journal of Systematic and Evolutionary Microbiology (2021) (See Supplementary 5).

CHAPTER 4. DISCUSSION

4.1. Potential of myxobacteria from Indonesian mangroves as antimicrobial agents

Myxobacteria are Gram-negative bacteria which are taxonomically included among the group of *Delta-proteobacteria* [33]. One of the economic benefits of myxobacteria is the ability to produce diverse bioactive metabolites.

Indonesian mangrove ecosystems are still poorly explored and only a small number of scientific reports are available for the production of antimicrobial metabolites especially from predatory bacteria such as myxobacteria [96]. This is the first report, which describes the characteristics of myxobacteria isolated from Indonesian mangrove ecosystems. These results provide new information about the ecology of myxobacteria and it will be useful for further studies of their novel compounds for antimicrobial activity.

Twenty-five strains were isolated from three different sites of Indonesian mangroves. Using conventional methods for isolation, *Myxococcus* sp. strains were found to be present in all samples. The fruiting bodies of *Myxococcus* sp. were easy to purify and cultivate under laboratory conditions [97].

Some rare myxobacteria, like *Chondromyces* sp. and *Racemicystis* sp. were isolated from Jakarta and Yogyakarta mangroves, respectively. However, our research may have limitations if we compare the myxobacterial diversity data from culture-independent methods (See 4.2) to the data of myxobacterial isolates from culture-dependent methods. There are several hypotheses for a low cultivation rate of myxobacteria in the present study. The presence of myxobacteria is not dominant in natural sources [31] and only 1 % of them has been isolated and characterized so far [98]. Some fungal or bacterial contaminants often overgrow samples before myxobacteria can be observed. Further, the growth requirements in the laboratory might be not suitable for the uncultivated myxobacterial species. Mohr *et al*. [37] also mentioned that the uncultured myxobacteria might represent rarely fruiting body forms in isolation medium and so are difficult to detect and isolate.

Twenty-five crude extracts from 25 strains were characterized for antimicrobial activities. Thirteen out of twenty-five crude extract yielded different activities against pathogenic microorganisms. Sharma *et al* mentioned that crude extracts with strong antimicrobial activities could hint to the presence of promising antimicrobial compounds against infectious disease and treatment [99]. Furthermore, these results were consistent with a previous study of Linares-Otoya *et al* [39] on antimicrobial screening of predatory bacteria from coastline. These predatory bacteria including myxobacteria from coastline may be promising sources for novel antibiotics [39].

In this study, we only focused on the active crude extract of *Racemicystis* sp. strain 503MSO, an isolate from Yogyakarta mangroves. *Racemicystis* sp. strain 503MSO was selected for further analysis because there was no information about potential bioactive compound from genus *Racemicystis* in myxobase database yet. Therefore, it is a big chance to get new compound from this genus.

The active fraction of *Racemicystis* sp. strain 503MSO, an isolate from Yogyakarta mangroves with *m/z* 375.2531 $[M+H]^+$ and 604.3847 $[M+H]^+$ were detected in this study and may represent so far, an undescribed compound. Therefore, *Racemicystis persica* strain 503MSO should be further explored including substantial work for optimizing isolation, structure elucidation and biological activity of the pure substance.

Mangrove habitats are a rich source for antimicrobial compounds produced by microorganism [35, 43, 45], highlighting of exploring their diversity. Myxobacteria from Indonesian mangroves have a huge potential to produce new anti-infectives. Therefore, isolation strategies of myxobacteria from neglected habitats such as mangroves are still extremely promising approaches for the detection of biological active compounds. Halotolerance techniques [46, 103, 104], diffusion chamber [105], or I-chip [106] could be used to improve isolation strategies of myxobacteria. Moreover, myxobacterial isolates from mangroves and their antimicrobial activity could be applied for further studies of other bioactivities against different "ESKAPE" [107] microbial pathogens such as *Enterococcus faecium*, *Klebsiella pneumonia* and *Enterobacter* species.

Overall, we believe that our work could be a starting point for further studies on myxobacteria with their diversity and unique community structures regarding their potential sources for new bioactive compounds.

This data will be reported in the Current Microbiology Journal (2021) (See Supplementary 6).

4.2. Evaluation of myxobacterial diversity by Illumina Miseq Sequencing

To analyze whether the standard isolation procedures in our lab are appropriate for the marine myxobacteria isolated from different areas of mangroves, we evaluated 16S rRNA genes of cultivable myxobacteria together with cultivation-independent metagenome analyses by Illumina sequencing. Indonesian mangroves explored in the present study were unexploited manifold marine habitats and have therefore a huge potential for the isolation of new uncommon species of myxobacteria.

Indonesian mangroves were inhabited by complex myxobacterial communities as shown by amplification using primer targeting *Cystobacterineae* (W2/R1525) and *Sorangiineae/Nannocystineae* (W5/R1525) [31, 36, 37]. Previously, Linares-Otoya *et al*. [39] observed that the use of universal primers (F515/R806) yielded only a neglectable amount of myxobacterial sequences (equivalent to 0.0002%) as also indicated by our work.

During our study, molecular survey (independent-cultivation) revealed that myxobacteria communities are more diverse than assumed. This lends support to the previous finding in the literature [31, 34, 36, 37, 108]. The diversity of myxobacteria obtained by using the NGS method has advantages compared to previous studies comprising DGGE (denaturing gel gradient electrophoresis) [109], hybridization analysis of the 16S rRNA gene library [31], or library analysis [36, 37]. By use of two PCR reactions (W2/R1525 and W5/R1525) an overview of all three suborders, i.e. *Cystobacterineae*, *Sorangiineae* and *Nannocystineae* could be obtained [110, 111] and insight into rare genera in complex communities such as mangroves could be achieved [104]. However, with the current method, a high

amount of non-*Myxococcales* genera were amplified and target sequences often comprised <10% of sequences. Evidently, optimization of primers is still necessary. Moreover, a species level information is only possible in exceptional cases using the V5V6 hypervariable regions as performed here.

This data will be reported in the Current Microbiology Journal (2021) (See Supplementary 6).

4.3. Multilocus sequences analysis (MLSA) of myxobacteria from mangrove

The most remarkable result from the data is that almost all type strains and myxobacteria isolates from mangroves could be amplified with at least one of the housekeeping genes. The results presented in this study are consistent with those of previous studies [27, 112, 113] which confirmed that MLSA technique is a powerful method to define relationship within and between species of bacteria.

Taken together, the 16S rRNA gene sequence analyses are hitherto fundamental to understand bacterial phylogeny and diversity, including uncultivable bacteria [114, 115]. Six of the housekeeping genes, *gyrB*, *lepA*, *fusA*, *pyrG*, *pgm* and *rpoB* were amplified to all type strains and myxobacteria isolates from mangroves. However, *lepA* and *fusA* genes were not included for MLSA analyses because strains 412MSO, 82MSO, 411MSO, 421MSO, 471MSO and 16MSO could not be amplified with *lepA* gene and all myxobacteria isolates also could not be able to be amplified with *fusA* gene as well. Phylogenetic tree of single housekeeping genes was not presented in this study. Glaeser & Kämpfer [27] mentioned that single housekeeping genes might distort the phylogeny tree because of the horizontal gene transfer and genetic recombination. Consequently, a single housekeeping gene only provides the evolution of single gene and could not reflect the “true” phylogenetic relationship. Furthermore, Hanage *et al.* [116] proposed that concatenation of loci enhanced taxonomic position by adding data that are more informative on species level or even strains.

The 16S rRNA gene sequencing had cut-off value for identification of to the species (98.70 %), genera (94.5 %), family (86.5 %), order (82.0 %), class (78.5 %) and phylum (75.0 %) level [117]. In contrast with 16S rRNA gene, MLSA scheme lacks in uniform cut-off value for identification of species level and a few reference sequences of myxobacteria based on the housekeeping genes has been mentioned in some public databases [118]. Approximately 93 % to 97 % of MLSA similarities were proposed to be as a threshold for within and between *Myxococcus* species delineation in this study. In contrast to this study, some of other bacteria revealed different similarities using MLSA technique. In *Streptomyces* sp. group, the concatenation of five genes give 0.007 of nucleotide sequences distances corresponding to 70 % of DDH value as the species cut-off for the whole genus [114]. *Thioclava* bacteria revealed 97.30 % of similarity to the proposed species level [119]. *Burkholderia* and *Bacillus* also defined 97 % and 97.74 % similarity to promote the species level, respectively [120].

The present study supports the idea that MLSA technique is an alternative tool for the phylogenetic analyses. However, MLSA technique has only investigated phylogenetic tree of the members of *Myxococcus* spp., which are isolated from Indonesian mangroves. Consequently, information from other genera could be required. Further studies should aim to compare and to correlate dDDH (digital DNA-DNA hybridization) with MLSA technique.

4.4. *Ohtaekwangia* sp. nov., a gliding bacterium isolated from Indonesian mangroves

This is the first report that describes a new species of gliding bacteria belonging to the genus *Ohtaekwangia* together with their potential for antimicrobial activity from unexplored Indonesian habitat, especially in the mangrove forest. *Ohtaekwangia* sp. strain 313MSO and strain 314MSO were isolated from Indonesian mangroves. Strain 313MSO with lower similarity 16S rRNA gene of 96.06 % than strain 314MSO was selected for in-depth polyphasic study. Further, Pablo *et al*. [117] suggested that the 16S rRNA gene sequences with similarity under of 98.70 % could be defined as new species.

Polyphasic study of strain 313MSO showed different characteristics compared to reference strains. For examples, strain 313MSO grows optimally on VY/2 agar, whereas references strains on R2A agar [75]. Further, the phylogenetic tree clearly exhibited separation from the reference strains. However, the determination of full genome sequences including G+C content [23], DNA-DNA hybridization [26] and quinone production [79] should be the subject of a further study.

Most of the crude extracts of strain 313MSO that was cultivated in 23 different media revealed specific inhibition towards *Staphylococcus aureus*. A previous study on antibacterial activity of aquatic gliding bacteria [121] mentioned that aquatic gliding bacteria had potential for the development of a broad spectrum anti-infectives. *Flavobacterium anhuiense* strain RPD001 for example, had activities against *Listeria monocytogenes* (MIC 150 µg/ml), *Staphylococcus aureus* (MIC 75 µg/ml) and *Vibrio cholerae* (MIC 300 µg/ml). However, the pure active compound that is responsible for the activity against these pathogenic microorganisms is not mentioned in their study.

Marinoquinolines A and its five derivates (B-F) were identified as products of our strain in this study. Despite of the fact that Okanya *et al.* [122] isolated the compounds from strain *Ohtaekwangia kribbensis* PWu25 in E medium, strain 313MSO had no antimicrobial activity in the same medium. Therefore, due to urgency of finding of biologically active new compounds, further experiment such as co-cultivation experiment [123, 124] will be required for stimulation of secondary metabolites of strain 313MSO.

In conclusion, mangroves are ecosystems that harbour gliding bacteria, since the area an unique environment between marine and terrestrial habitats. Strain 313MSO is classified as a new species with different characteristics within the genus *Ohtaekwangia.* Its crude extracts showed specific antimicrobial activities against tested Gram-positive pathogenic bacteria.

This data will be reported in the Hayati Journal of Biosciences (2020) **(Submitted)** (See Supplementary 7).

CHAPTER 5. CONCLUSION and OUTLOOK

5.1. Conclusion

Indonesian mangroves are an interesting habitat of predatory and other gliding bacteria as potential producers of natural products. Two methods, the traditional isolation way (dependent cultivation) and the molecular survey (independent cultivation) were carried out in this study. The cultivation independent method by NGS with specific myxobacteria primer revealed sequences of members of the order *Myxococcales*. However, dependent cultivation of myxobacteria drawn less abundances than independent cultivation method and revealed 27 predatory bacteria including 25 myxobacteria and 2 gliding bacteria.

Polyphasic study of predatory bacteria clarified the characteristics of strain 313MSO compared to type strains of the genus *Ohtaekwangia*. Furthermore, the concatenated six housekeeping genes clearly sketched position taxa within and between myxobacterial species.

Screen on 48 crude extracts was carried out to characterize antimicrobial activity from predatory bacteria including 25 crude extracts of myxobacteria and 23 crude extracts of other gliding bacteria, respectively. Thirteen crude extracts of myxobacteria had moderate activities against at least one of human pathogenic microorganisms compared to other gliding bacteria, strain 313MSO as well. Further, crude extract of *Racemycistis persica* strain 503MSO indicated a novel compound.

5.2. Outlook

Diversity and antimicrobial potential of predatory bacteria such as myxobacteria and other gliding bacteria were successfully drawn at Indonesian mangroves. Research strategies such as NGS method, specific primer, polyphasic taxonomic approach, growth inhibition test using a serial dilution test for antimicrobial activity were selected to achieve our goals in this study.

Some of public databases such as NCBI, EMBL, RDP, in-house myxobase database and bioinformatic tools also support our study. Numerous predatory bacterial strains were obtained and their activity described potential of predatory bacteria against human pathogenic microorganisms.

Finally, mangrove habitats are a rich source for antimicrobial compounds produced by myxobacteria highlighting the necessity to explore their diversity.

REFERENCES

1. **Rončević T, Puizina J, Tossi A**. Antimicrobial peptides as anti-infective agents in pre-post-antibiotic era? *Int J Mol Sci*;20. Epub ahead of print 2019. DOI: 10.3390/ijms20225713.

2. **Luo Y, Cobb RE, Zhao H**. Recent advances in natural product discovery. *Curr Opin Biotechnol* 2014;30:230–237.

3. **Dworkin M**. Myxobacteria. In: *Encyclopedia of Life Sciences*. pp. 1–5.

4. **Reichenbach H**. The ecology of the myxobacteria. *Environ Microbiol* 1999;1:15–21.

5. **Dawid W**. Biology and global distribution of myxobacteria in soils. *FEMS Microbiology Reviews* 2000;24:403–427.

6. **Mohr K**. Diversity of Myxobacteria—We Only See the Tip of the Iceberg. *Microorganisms* 2018;6:84.

7. **Forli S**. Epothilones: From discovery to clinical trials. *Curr Top Med Chem* 2014;14:2312–2321.

8. **Borzilleri RM, Zheng X, Schmidt RJ, Johnson JA, Kim S-H, *et al.*** A Novel Application of a Pd(0)-Catalyzed Nucleophilic Substitution Reaction to the Regio- and Stereoselective Synthesis of Lactam Analogues of the Epothilone Natural Products. *J Am Chem Soc* 2000;122:8890–8897.

9. **Kavallaris M**. Microtubules and resistance to tubulin-binding agents. *Nat Rev Cancer* 2010;10:194–204.

10. **Gerth K, Pradella S, Perlova O, Beyer S, Müller R**. Myxobacteria: Proficient producers of novel natural products with various biological activities - Past and future biotechnological aspects with the focus on the genus *Sorangium*. *Journal of Biotechnology* 2003;106:233–253.

11. **Wenzel SC, Müller R**. Myxobacteria—'microbial factories' for the production of bioactive secondary metabolites. *Mol Biosyst* 2009;5:567.

12. **Landwehr W, Wolf C, Wink J**. Actinobacteria and Myxobacteria---Two of the Most Important Bacterial Resources for Novel Antibiotics. In: Stadler M, Dersch P (editors). *How to Overcome the Antibiotic Crisis : Facts, Challenges, Technologies and Future Perspectives*. Cham: Springer International Publishing. pp. 273–302.

13. **Mulwa L, Stadler M**. Antiviral Compounds from Myxobacteria. *Microorganisms* 2018;6:73.

14. **Hoffmann T, Krug D, Bozkurt N, Duddela S, Jansen R, *et al.*** Correlating

chemical diversity with taxonomic distance for discovery of natural products in myxobacteria. *Nat Commun* 2018;9:1–10.

15. **Schäberle TF, Lohr F, Schmitz A, König GM**. Antibiotics from myxobacteria. *Nat Prod Rep* 2014;31:953.

16. **Reichenbach H**. *The Order Cytophagales*. 1992. Epub ahead of print 1992. DOI: 10.1007/978-1-4757-2191-1_37.

17. **Reichenbach H**. Taxonomy of the Gliding Bacteria. *Annu Rev Microbiol* 1981;35:339–364.

18. **Katayama N, Nozaki Y, Okonogi K, Ono H, Harada S, *et al.*** Formadicins, new monocylic b-lactam antibiotics of bacterial origin. *J Antibiot* 1985;39:1117–1127.

19. **Ikegami Y, Takeuchi N, Hanada M, Hasegawa Y, Ishii K, *et al.*** Topostin, a novel inhibitor of mammalian DNA topoisomerase I from Flexibacter topostinus sp. nov. II. Purification and some properties of topostin. *J Antibiot (Tokyo)* 1990;43:158–162.

20. **Singh PD, Ward PC, Scott Wells J, Ricca CM, Trejo WH, *et al.*** Bacterial Production of Deacetoxycephalosporin C. *J Antibiot (Tokyo)* 1982;35:1397–1399.

21. **Park JH, Kim R, Aslam Z, Jeon CO, Chung YR**. Lysobacter capsici sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus *Lysobacter. Int J Syst Evol Microbiol* 2008;58:387–392.

22. **Okanya PW, Mohr KI, Gerth K, Jansen R, Müller R**. Marinoquinolines A-F, pyrroloquinolines from *Ohtaekwangia kribbensis* (Bacteroidetes). *J Nat Prod* 2011;74:603–608.

23. **Prakash O, Verma M, Sharma P, Kumar M, Kumari K, *et al.*** Polyphasic approach of bacterial classification - An overview of recent advances. *Indian J Microbiol* 2007;47:98–108.

24. **Das S, Dash HR, Mangwani N, Chakraborty J, Kumari S**. Understanding molecular identification and polyphasic taxonomic approaches for genetic relatedness and phylogenetic relationships of microorganisms. *J Microbiol Methods* 2014;103:80–100.

25. **Quainoo S, Coolen JPM, van Hijum SAFT, Huynen MA, Melchers WJG, *et al.*** Whole-Genome Sequencing of Bacterial Pathogens: the Future of Nosocomial Outbreak Analysis. *Clin Microbiol Rev* 2017;30:1015–1063.

26. **Sentausa E, Fournier P-E**. Advantages and limitations of genomics in prokaryotic taxonomy. *Clin Microbiol Infect* 2013;19:790–795.

27. **Glaeser SP, Kämpfer P**. Multilocus sequence analysis (MLSA) in prokaryotic taxonomy. *Syst Appl Microbiol* 2015;38:237–245.

28. **Das S, Dash HR, Mangwani N, Chakraborty J, Kumari S**. Understanding molecular identification and polyphasic taxonomic approaches for genetic relatedness and phylogenetic relationships of microorganisms. *J Microbiol Methods* 2014;103:80–100.

29. **HOOK LA, LARKIN JM, BROCKMAN ER**. Isolation, Characterization, and Emendation of Description of *Angiococcus disciformis* (Thaxter 1904) Jahn 1924 and Proposal of a Neotype Strain. *Int J Syst Evol Microbiol* 1980;30:135–142.

30. **Iizuka T, Yamanaka S, Nishiyama T, Hiraishi A**. Isolation and phylogenetic analysis of aerobic copiotrophic ultramicrobacteria from urban soil. *J Gen Appl Microbiol* 1998;44:75–84.

31. **Wu ZH, Jiang DM, Li P, Li YZ**. Exploring the diversity of myxobacteria in a soil niche by myxobacteria-specific primers and probes. *Environ Microbiol* 2005;7:1602–1610.

32. **Brinkhoff T, Fischer D, Vollmers J, Voget S, Beardsley C, *et al.*** Biogeography and phylogenetic diversity of a cluster of exclusively marine myxobacteria. *ISME J* 2012;6:1260–1272.

33. **Zhang X, Yao Q, Cai Z, Xie X, Zhu H**. Isolation and Identification of Myxobacteria from Saline-Alkaline Soils in Xinjiang, China. *PLoS One* 2013;8:e70466.

34. **Li S guang, Zhou X wen, Li P fei, Han K, Li W, *et al.*** The existence and diversity of myxobacteria in lake mud - a previously unexplored myxobacteria habitat. *Environ Microbiol Rep* 2012;4:587–595.

35. **Li F, Liu S, Lu Q, Zheng H, Osterman IA, *et al.*** Studies on antibacterial activity and diversity of cultivable actinobacteria isolated from mangrove soil in futian and maoweihai of China. *Evidence-based Complement Altern Med*;2019. Epub ahead of print 2019. DOI: 10.1155/2019/3476567.

36. **Mohr KI, Stechling M, Wink J, Wilharm E, Stadler M**. Comparison of myxobacterial diversity and evaluation of isolation success in two niches: Kiritimati Island and German compost. *Microbiologyopen* 2016;5:268–278.

37. **Mohr KI, Zindler T, Wink J, Wilharm E, Stadler M**. Myxobacteria in high moor and fen: An astonishing diversity in a neglected extreme habitat. *Microbiologyopen* 2017;6:1–9.

38. **Escobar-Zepeda A, Vera-Ponce de León A, Sanchez-Flores A**. The Road to Metagenomics: From Microbiology to DNA Sequencing Technologies and Bioinformatics. *Front Genet* 2015;6:348.

39. **Linares-Otoya L, Linares-Otoya V, Armas-Mantilla L, Blanco-Olano C, Crüsemann M, *et al.*** Diversity and antimicrobial potential of predatory bacteria from the peruvian coastline. *Mar Drugs* 2017;15:1–14.

40. **Earp HS, Prinz N, Cziesielski MJ, Andskog M**. For a World Without Boundaries: Connectivity Between Marine Tropical Ecosystems in Times of Change. In: Jungblut S, Liebich V, Bode M (editors). *YOUMARES 8 -- Oceans Across Boundaries: Learning from each other*. Cham: Springer International Publishing; 2018. pp. 125–144.

41. **Gomes NCM, Cleary DFR, Calado R, Costa R**. Mangrove bacterial richness. *Commun Integr Biol* 2011;4:419–423.

42. **Michelato Ghizelini A, Cristina Santana Mendonça-Hagler L, Macrae A**. Microbial diversity in brazilian mangrove sediments - A mini review. *Brazilian J Microbiol* 2012;43:1242–1254.

43. **Thatoi H, Behera BC, Mishra RR, Dutta SK**. Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: A review. *Ann Microbiol* 2013;63:1–19.

44. **Isaka M, Suyarnsestakorn C, Tanticharoen M, Kongsaeree P, Thebtaranonth Y**. Aigialomycins A-E, new resorcylic macrolides from the marine mangrove fungus *Aigialus parvus*. *J Org Chem* 2002;67:1561–1566.

45. **Retnowati W**. Identification of *Streptomyces* sp-MWS1 Producing Antibacterial Compounds. *Indones J Trop Infect Dis* 2010;1:82.

46. **Iizuka T, Jojima Y, Hayakawa A, Fujii T, Yamanaka S, *et al.*** *Pseudenhygromyxa salsuginis* gen. nov., sp. nov., a myxobacterium isolated from an estuarine marsh. *Int J Syst Evol Microbiol* 2013;63:1360–1369.

47. **Jiang XT, Peng X, Deng GH, Sheng HF, Wang Y, *et al.*** Illumina Sequencing of 16S rRNA Tag Revealed Spatial Variations of Bacterial Communities in a Mangrove Wetland. *Microb Ecol* 2013;66:96–104.

48. **Murdiyarso D, Purbopuspito J, Kauffman JB, Warren MW, Sasmito SD, *et al.*** The potential of Indonesian mangrove forests for global climate change mitigation. *Nat Clim Chang* 2015;5:1089–1092.

49. **Sidik F, Supriyanto B, Krisnawati H, Muttaqin MZ**. Mangrove conservation for climate change mitigation in Indonesia. *WIREs Clim Chang* 2018;9:e529.

50. **Brosius J, Palmer ML, Kennedy PJ, Noller HF**. Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli. *Proc Natl Acad Sci* 1978;75:4801–4805.

51. **Rath S, Heidrich B, Pieper DH, Vital M**. Uncovering the trimethylamine-producing bacteria of the human gut microbiota. *Microbiome* 2017;5:1–14.

52. **Schulz C, Schütte K, Koch N, Vilchez-Vargas R, Wos-Oxley ML, *et al.*** The active bacterial assemblages of the upper GI tract in individuals with and without Helicobacter infection. *Gut* 2018;67:216–225.

53. **Wang Q, Garrity GM, Tiedje JM, Cole JR**. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 2007;73:5261–5267.

54. **Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, *et al.*** Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 2009;75:7537–7541.

55. **Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, *et al.*** The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;41:D590-6.

56. **Webb GI**. Naïve Bayes. In: Sammut C, Webb GI (editors). *Encyclopedia of Machine Learning*. Boston, MA: Springer US. pp. 713–714.

57. **Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E**. *The Prokaryotes - Delta and Epsilon Subclasses. Deeply Rooting Bacteria*. Epub ahead of print 2006. DOI: 10.1007/0-387-30747-8.

58. **Hall TA**. BIOEDIT: A USER-FRIENDLY BIOLOGICAL SEQUENCE ALIGNMENT EDITOR AND ANALYSIS PROGRAM FOR WINDOWS 95/98/ NT. 1999.

59. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ**. Basic local alignment search tool. *J Mol Biol* 1990;215:403–410.

60. **Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M**. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.

61. **Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M**. Highly parallelized inference of large genome-based phylogenies. *Concurr Comput Pract Exp* 2014;26:1715–1729.

62. **Edgar RC**. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792–1797.

63. **Stamatakis A**. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.

64. **Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A**. How many bootstrap replicates are necessary? *J Comput Biol* 2010;17:337–354.

65. **Swofford D**. PAUP*: Phylogenetic analysis using parsimony (*and other

methods), Version 4.0b10. 2002.

66. **Wibowo JT, Kellermann MY, Versluis D, Putra MY, Murniasih T, *et al.*** Biotechnological Potential of Bacteria Isolated from the Sea Cucumber *Holothuria leucospilota* and *Stichopus vastus* from Lampung , Indonesia.

67. **Mueller JH, Hinton J**. A Protein-Free Medium for Primary Isolation of the Gonococcus and Meningococcus. *Proc Soc Exp Biol Med* 1941;48:330–333.

68. **Mycosel BBL, Deep A, Agar M**. BBL ™ Mycosel ™ Agar (Deep Fill). 2006;6–7.

69. **Krug D, Müller R**. Secondary metabolomics: The impact of mass spectrometry-based approaches on the discovery and characterization of microbial natural products. *Nat Prod Rep* 2014;31:768–783.

70. **Babicki S, Arndt D, Marcu A, Liang Y, Grant JR, *et al.*** Heatmapper: web-enabled heat mapping for all. *Nucleic Acids Res* 2016;44:W147-53.

71. **Gu Z, Eils R, Schlesner M**. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 2016;32:2847–2849.

72. **Korbie DJ, Mattick JS**. Touchdown PCR for increased specificity and sensitivity in PCR amplification. *Nat Protoc* 2008;3:1452–1456.

73. **Kumar S, Stecher G, Li M, Knyaz C, Tamura K**. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–1549.

74. **Librado P, Rozas J**. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 2009;25:1451–1452.

75. **Yoon JH, Kang SJ, Lee SY, Lee JS, Park S**. *Ohtaekwangia koreensis* gen. nov., sp. nov. and *Ohtaekwangia kribbensis* sp. nov., isolated from marine sand, deep-branching members of the phylum Bacteroidetes. *Int J Syst Evol Microbiol* 2011;61:1066–1072.

76. **Humble MW, King A, Phillips I**. API ZYM: a simple rapid system for the detection of bacterial enzymes. *J Clin Pathol* 1977;30:275–277.

77. **Huysmans MB, Turnidge JD, Williams JH**. Evaluation of API Campy in comparison with conventional methods for identification of thermophilic campylobacters. *J Clin Microbiol* 1995;33:3345–3346.

78. **Bauer AW, Kirby WM, Sherris JC, Turck M**. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966;45:493–496.

79. **Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, *et***

al. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.

80. **Collins MD, Shah HN**. Fatty acid, menaquinone and polar lipid composition of *Rothia dentocariosa*. *Arch Microbiol* 1984;137:247–249.

81. **Gadagkar SR, Rosenberg MS, Kumar S**. Inferring species phylogenies from multiple genes: Concatenated sequence tree versus consensus gene tree. *J Exp Zool Part B Mol Dev Evol* 2005;304B:64–74.

82. **Ludwig W, Schleifer KH**. Bacterial phylogeny based on 16S and 23S rRNA sequence analysis. *FEMS Microbiol Rev* 1994;15:155–173.

83. **Hug LA, Baker BJ, Anantharaman K, Brown CT, Probst AJ, *et al.*** A new view of the tree of life. *Nat Microbiol* 2016;1:16048.

84. **Mohr KI, Wolf C, Nübel U, Szafrańska AK, Steglich M, *et al.*** A polyphasic approach leads to seven new species of the cellulose-decomposing genus *Sorangium*, *Sorangium ambruticinum* sp. nov., *Sorangium arenae* sp. nov., *Sorangium bulgaricum* sp. nov., *Sorangium dawidii* sp. nov., *Sorangium kenyense* sp. nov., *Sorangium* or. *Int J Syst Evol Microbiol* 2018;68:3576–3586.

85. **Meier-Kolthoff JP, Klenk H-P, Göker M**. Taxonomic use of DNA G+C content and DNA–DNA hybridization in the genomic age. *Int J Syst Evol Microbiol* 2014;64:352–356.

86. **Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, *et al.*** Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.

87. **Richter M, Rosselló-Móra R**. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* 2009;106:19126–19131.

88. **Yoon JH, Kang SJ, Lee SY, Lee JS, Park S**. *Ohtaekwangia koreensis* gen. nov., sp. nov. and *Ohtaekwangia kribbensis* sp. nov., isolated from marine sand, deep-branching members of the phylum Bacteroidetes. *Int J Syst Evol Microbiol* 2011;61:1066–1072.

89. **Kim J-J, Alkawally M, Brady AL, Rijpstra WIC, Sinninghe Damsté JS, *et al.*** *Chryseolinea serpens* gen. nov., sp. nov., a member of the phylum Bacteroidetes isolated from soil. *Int J Syst Evol Microbiol* 2013;63:654–660.

90. **Maejima Y, Iino T, Muraguchi Y, Fukuda K, Ohkuma M, *et al.*** *Chryseotalea sanaruensis* gen. nov., sp., nov., a Member of the Family *Cytophagaceae*, Isolated from a Brackish Lake in Hamamatsu Japan. *Curr Microbiol* 2020;77:306–312.

91. **Jones DM**. Manual of Methods for General Bacteriology. *Journal of Clinical Pathology* 1981;34:1069.

92. **Komagata K, Suzuki K-I**. 4 Lipid and Cell-Wall Analysis in Bacterial Systematics. In: Colwell RR, Grigorova R (editors). *Current Methods for Classification and Identification of Microorganisms*. Academic Press. pp. 161–207.

93. **Lechevalier MP, De Bievre C, Lechevalier H**. Chemotaxonomy of aerobic Actinomycetes: Phospholipid composition. *Biochem Syst Ecol* 1977;5:249–260.

94. **Sasser M**. Technical Note # 101 M icrobial Identification by Gas Chromatograph ic Analysis of Fatty Acid Methyl Esters (GC-FAME). *Tech Note* 2009;1–6.

95. **Albers SV, Siebers B**. The family *Sulfolobaceae*. 2014. Epub ahead of print 2014. DOI: 10.1007/978-3-642-38954-2_329.

96. **Liu Y, Yao Q**. Meta-16S rRNA gene phylogenetic reconstruction reveals astonishing diversity of the cosmopolitan myxobacteria Yang Liu.

97. **Zhang X, Yao Q, Cai Z, Xie X, Zhu H**. Isolation and Identification of Myxobacteria from Saline-Alkaline Soils in Xinjiang, China. *PLoS One* 2013;8:1–11.

98. **Muyzer G**. DGGE/TGGE a method for identifying genes from natural ecosystems. *Curr Opin Microbiol* 1999;2:317–322.

99. **Sharma P, Thakur D**. Antimicrobial biosynthetic potential and diversity of culturable soil actinobacteria from forest ecosystems of Northeast India. *Sci Rep* 2020;10:1–18.

100. **Zhang Q, Li S, Chen Y, Tian X, Zhang H, *et al.*** New diketopiperazine derivatives from a deep-sea-derived *Nocardiopsis alba* SCSIO 03039. *J Antibiot (Tokyo)* 2013;66:31–36.

101. **Mangamuri UK, Muvva V, Poda S, Manavathi B, Bhujangarao C, *et al.*** Chemical characterization & bioactivity of diketopiperazine derivatives from the mangrove derived *Pseudonocardia endophytica. Egypt J Aquat Res* 2016;42:169–175.

102. **Ding Y, Zhu X, Hao L, Zhao M, Hua Q, *et al.*** Bioactive Indolyl Diketopiperazines from the Marine Derived Endophytic *Aspergillus versicolor* DY180635. *Mar Drugs*;18. Epub ahead of print 2020. DOI: 10.3390/md18070338.

103. **Iizuka T, Jojima Y, Fudou R, Tokura M, Hiraishi A, *et al.*** *Enhygromyxa salina* gen. nov., sp. nov., a slightly halophilic myxobacterium isolated from the coastal areas of Japan. *Syst Appl Microbiol* 2003;26:189—196.

104. **Fudou R, Jojima Y, Iizuka T, Yamanaka S**. *Haliangium ochraceum* gen. nov., sp. nov. and *Haliangium tepidum* sp. nov.: novel moderately halophilic myxobacteria isolated from coastal saline environments. *J Gen Appl Microbiol* 2002;48:109–116.

105. **Kaeberlein T, Lewis K, Epstein SS**. Science-2002-Kaeberlein. 2002;296:1127–1130.

106. **Nichols D, Cahoon N, Trakhtenberg EM, Pham L, Mehta A, *et al.*** Use of ichip for high-throughput in situ cultivation of 'uncultivable' microbial species. *Appl Environ Microbiol* 2010;76:2445–2450.

107. **De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, *et al.*** Antimicrobial resistance in ESKAPE pathogens. *Clin Microbiol Rev* 2020;33:1–49.

108. **Jiang DM, Wu ZH, Zhao JY, Li YZ**. Fruiting and non-fruiting myxobacteria: A phylogenetic perspective of cultured and uncultured members of this group. *Mol Phylogenet Evol* 2007;44:545–552.

109. **Li B, Yao Q, Zhu H**. Approach to analyze the diversity of myxobacteria in soil by semi-nested PCR-denaturing gradient gel electrophoresis (DGGE) based on taxon-specific gene. *PLoS One* 2014;9:1–8.

110. **Fanning S, Proos S, Jordan K, Srikumar S**. A review on the applications of next generation sequencing technologies as applied to food-related microbiome studies. *Front Microbiol* 2017;8:1–16.

111. **Black JS, Salto-Tellez M, Mills KI, Catherwood MA**. The impact of next generation sequencing technologies on haematological research - A review. *Pathogenesis* 2015;2:9–16.

112. **Delamuta JRM, Ribeiro RA, Menna P, Bangel EV, Hungria M**. Multilocus sequence analysis (MLSA) of Bradyrhizobium strains: Revealing high diversity of tropical diazotrophic symbiotic bacteria. *Brazilian J Microbiol* 2012;43:698–710.

113. **Lai Q, Liu Y, Yuan J, Du J, Wang L, *et al.*** Multilocus sequence analysis for assessment of phylogenetic diversity and biogeography in Thalassospira bacteria from diverse marine environments. *PLoS One* 2014;9:e106353.

114. **Rong X, Huang Y**. *Multi-locus sequence analysis. Taking prokaryotic systematics to the next level*. 1st ed. Elsevier Ltd. Epub ahead of print 2014. DOI: 10.1016/bs.mim.2014.10.001.

115. **López-Hermoso C, de la Haba RR, Sánchez-Porro C, Papke RT, Ventosa A**. Assessment of MultiLocus Sequence Analysis As a Valuable Tool for the Classification of the Genus Salinivibrio. *Front Microbiol* 2017;8:1107.

116. **Hanage WP, Kaijalainen T, Herva E, Saukkoriipi A, Syrjänen R, *et al.*** Using Multilocus Sequence Data To Define the Pneumococcus. *J Bacteriol* 2005;187:6223 LP – 6230.

117. **Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, *et al.*** Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* 2014;12:635–645.

118. **Stackebrandt E, Päuker O, Steiner U, Schumann P, Sträubler B, *et al.*** Taxonomic characterization of members of the genus *Corallococcus:* Molecular divergence versus phenotypic coherency. *Syst Appl Microbiol* 2007;30:109–118.

119. **Liu Y, Lai Q, Shao Z**. A multilocus sequence analysis scheme for phylogeny of Thioclava bacteria and proposal of two novel species. *Front Microbiol* 2017;8:1–11.

120. **Peeters C, Zlosnik JEA, Spilker T, Hird TJ, LiPuma JJ, *et al.*** *Burkholderia pseudomultivorans* sp. nov., a novel Burkholderia cepacia complex species from human respiratory samples and the rhizosphere. *Syst Appl Microbiol* 2013;36:483–489.

121. **Sangnoi Y, Anantapong T, Kanjana-Opas A**. Antibacterial activity of aquatic gliding bacteria. *Springerplus* 2016;5:116.

122. **Okanya PW, Mohr KI, Gerth K, Jansen R, Müller R**. Isolation and structure elucidation of secondary metabolites from the gliding bacteria *Ohtaekwangia kribbensis* and *Hyalangium minutum. J Nat Prod* 2011;74:603–608.

123. **Bertrand S, Bohni N, Schnee S, Schumpp O, Gindro K, *et al.*** Metabolite induction via microorganism co-culture: a potential way to enhance chemical diversity for drug discovery. *Biotechnol Adv* 2014;32:1180–1204.

124. **Marmann A, Aly AH, Lin W, Wang B, Proksch P**. Co-cultivation--a powerful emerging tool for enhancing the chemical diversity of microorganisms. *Mar Drugs* 2014;12:1043–1065.

SUPPLEMENTARY

Table S1. Accession number of Mangroves samples (PRJNA678217)

Location	Samples	Accession Number
MT	M39_W2	SRX9502207
	M39_W5	SRX9502208
	M41_W2	SRX9502211
	M41_W5	SRX9502212
MA	M44_W2	SRX9502213
	M44_W5	SRX9502214
	M45_W2	SRX9502215
	M45_W5	SRX9502216
MK	M48_W2	SRX9502217
	M48_W5	SRX9502218
	M52_W2	SRX9502209
	M52_W5	SRX9502210

Figure S2. Structure of compound and their identification in crude extract of *Racemicystis* sp. strain 503MSO.

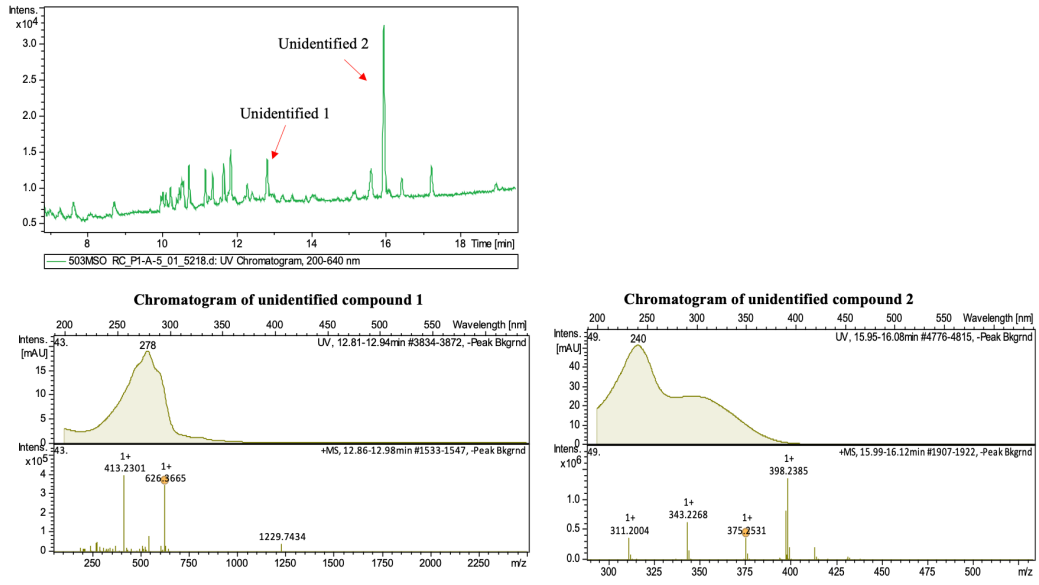
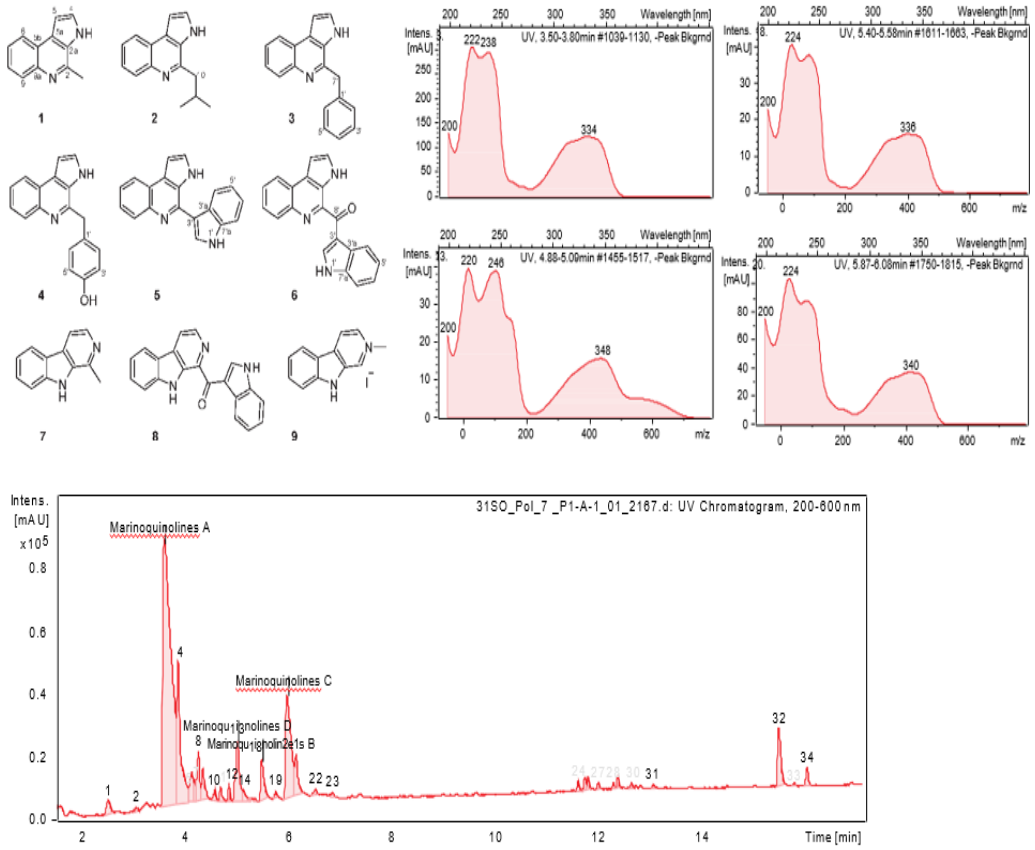


Table S3. List of accession number of predatory bacteria isolated from Indonesian Mangroves with housekeeping genes

No.	Code	Species	gyrB	lepA	fusA	rpoB	pyrG	Pgm
1	82MSO	*Corallococcus coraloides*	-	-	-	-	MW220098	-
2	412MSO	*Corallococcus coraloides*	MW220069	-	-	MW220110	-	-
3	411MSO	*Myxococcus fulvus*	-	-	-	MW220111	-	MW220086
4	191MSO	*Myxococcus fulvus*	MW220071	MW220083	-	MW220106	MW220096	MW220087
5	35MSO	*Myxococcus fulvus*	-	MW220081	-	MW220112	-	MW220084
6	471MSO	*Myxococcus macrosporus*	MW220074	-	-	MW220104	MW220095	MW220091
7	161MSO	*Myxococcus macrosporus*	MW220070	MW220080	-	MW220109	-	MW220085
8	521MSO	*Myxococcus macrosporus*	MW220072	MW220079	-	MW220103	MW220093	MW220089
9	421MSO	*Myxococcus macrosporus*	MW220075	-	-	MW220105	MW220094	MW220090
10	21MSO	*Myxococcus macrosporus*	MW220073	MW220082	-	MW220102	MW220092	MW220088
11	16MSO	*Nannocystis pusila*	-	-	-	MW220108	MW220097	-
12	112MSO	*Nannocystis pusila*	-	-	-	MW220107	-	-
13	182MSO	*Nannocystis pusila*	-	-	-	-	-	-
14	61MSO	*Chondromyces pediculatus*	-	MW220077	-	-	MW220099	-
15	41MSO	*Chondromyces robustus*	-	MW220076	-	-	MW220100	-
16	151MSO	*Chondromyces robustus*	-	MW220078	-	MW220113	MW220101	-

Figure S4. Structure of Marinoquinolines A and their derivatives compound [122] in crude extract of *Ohtaekwangia* sp. strain 313MSO.



Supplementary 5

Four new members of the family *Cytophagaceae*: *Cryseosolum histidinii* gen. nov., sp. nov., *Cryseosolum indiensis* gen. nov., sp. nov., *Reichenbachia cretensis*, gen. nov., sp. nov., and *Reichenbachia soli,* gen. nov., sp. nov. isolated from soil and animal dung

Author names
Senlie Octaviana[1,2*], Stefan Lorenczyk[3*], Frederike Ackert[1], Ulrich Nübel[4], Joachim Wink[1**]

Affiliation
[1]Microbial Strain Collection, Helmholtz Center for Infection Research, Inhoffenstraße 7, Braunschweig, 38124, Germany
[2]Research Center for Biotechnology, Indonesian Institute of Sciences, Cibinong, 16911, Indonesia
[3]Technical University of Braunschweig, Faculty of Mechanical Engineering, Schleinitzstraße 20, 38106 Braunschweig, Germany
[4]Leibniz Institute DSMZ, Braunschweig, Inhoffenstraße 7B, D-38124 Braunschweig, Germany

*equal work

Corresponding author
**Joachim Wink, Joachim.wink@helmholtz-hzi.de

Keyword
Cytophagaceae, diverse habitat, gliding bacteria

Repositories:
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strain PWU4^T, PWU5 ^{T}, PWU20^T and PWU37^T are MW182516, MW182517, MW182518 and MW182519. The GenBank/EMBL/DDBJ accession number for the draft genome sequences of strain PWU4^T, PWU5 ^{T}, PWU20^T and PWU37^T are ERR4837147, ERR4837148, ERR4837149, and ERR4837150 (Project number ERP124865).

ABSTRACT
From four strains isolated, PWU4^T and PWU20^T were from soil in Germany, strain PWU5 ^{T} was from soil in India, while PWU37^T was from a faces sheep collected in Crete Island. Cells were Gram-negative, strictly aerobic and rod shaped. They grew optimally between 28^{o}C and 34 ^{o}C, pH between 7.0 and 8.0 and without the addition of NaCl. Colonies were irregular and yellow. All the strains were catalase and oxidase-negative and grew on most mono- and disaccharides, a few

polysaccharides and organic acid. The predominant menaquinone was MK-7 and the major polar lipid were composed of phosphatidylethanolamine. Major fatty acid was $c_{16:1}$ ω7c in strain PWU4^T and PWU20^T, while $c_{16:1}$ ω5c in strain PWU5^T and PWU37^T. The DNA G+C content of strain PWU4^T; PWU5^T; PWU20 ^{T}; and PWU37^T were 50.2 mol %; 51.6 mol %; 39.8 mol % and 53.8 mol %, respectively. The 16S rRNA gene sequence analysis revealed that the closest relatives of strains PWU4^T, PWU5 ^{T}, PWU20^T and PWU37^T are less than 93.8 % compared to *Ohtaekwangia koreensis* 3B-2^T and *Ohtaekwangia kribbensis* 10AO^T. Furthermore, 16S rRNA gene sequence analysis among the strains classified them in two groups, where strain PWU4^T and PWU20^T shared 93.0 % sequence similarity, and strain PWU5^T and PWU37^T 97.5 %. However, in both groups represent different species on the low average nucleotide identity (ANI) of their genomes of strain PWU4^T and PWU20^T 69.7 % and strain PWU5^T and PWU37^T 83.8 %. Therefore, we proposed that the four strains represent four novel species of two new genera in the family *Cytophagaceae*. The type species of the novel genus *Cryseosolum* are *Cryseosolum histdinii* gen. nov., sp. nov. strain PWU4^T (=DSM 111594^T = NCCB 100798^T), *Cryseosolum indiensis* sp. nov. strain PWU20^T (=DSM 111597^T = NCCB 100800^T), and the type species of the novel genus *Reichenbachia* are *Reichenbachia cretensis* gen. nov., sp. nov. strain PWU5^T (=DSM 111596^T = NCCB 100799^T), *Reichenbachia soli* sp. nov. strain PWU37^T (=DSM 111595^T = NCCB 100801^T).

Introduction

The family *Cytophagaceae* was originally introduced in 1940 by Stanier [1] and it was the largest family within the single order *Cytophagales* with at least 25 genera and 80 species [2]. Isolates of the family *Cytophagaceae* are Gram-negative bacteria, chemoorganotrophic, aerobes but also a few anaerobes [3]. Furthermore, they are widely distributed in nature such as soil [4–6], freshwater [7, 8], airborne [9], dessert [10] and even glacier field [11].

Since the antibiotic golden age era, the phylum *Bacteriodetes* including the classes *Flavobacteriia* and *Cytophagia* contributed as producers of antimicrobial bioactive compounds [12–16]. During the screening of antimicrobial activity from the culture collection of Reichenbach at the Helmholtz Center for Infection Research (HZI) Germany, four novel bacterial strains (designated PWU4^T, PWU5^T, PWU20^T and PWU37^T) were identified herein. The aim of the present study was to explore the taxonomic status of the four bacteria as novel species by using the polyphasic approach.

Isolation and Ecology

Strain PWU4^T, PWU5 ^{T}, PWU20 ^{T} and PWU37 ^{T} were obtained from the microbial culture collection group at Helmholtz Center for Infection Research (HZI) Germany. Strain PWU4^T, PWU20 ^{T} and PWU37 ^{T} were revealed from soil samples collected in May 1990 at Braunschweig, Germany (52.22090 N 10.50902 E), in May 1989 at Lucknow, Uttar Pradesh, India (26.8684 N 80.90979 E) and in

September 1991 at Braunschweig (52.21501 N 10.53329 E), respectively. Strain PWU5^T was revealed from a face of sheep with plant residues collected in July 1988 at Crete Island (35.2463 N 25.09705 E).

The four strains were isolated using a dilution method on agar plates following the protocol of Reichenbach [17], maintained in E medium and kept directly with this medium at -80^{o}C for long-term preservation. For physiological and chemotaxonomy test, the four strains were grown without NaCl in E broth medium at their optimum pH and temperatures including pH 7 and 30^{o}C for strain PWU4^T, pH 7 and 28^{o}C for strain PWU20^T and strain PWU5^T, pH 7.4-8.0 and 34^{o}C for strain PWU37^T. *Ohtaekwangia koreensis* (3B-2^T) and *Ohtaekwangia kribbensis* (10AO^T) were used as references strains and were grown under the same culture conditions.

16S RNA phylogeny

The DNA of cultures were extracted using an Invisorb Spin Plant mini kit (Stratec Molecular, Germany). The GGDC web server [18], available at http://ggdc.dsmz.de/ was used to infer their phylogenetic relationships [19]. Briefly, after creating a multiple sequence alignment with MUSCLE [20], maximum likelihood and maximum parsimony trees were inferred with RAxML [21] and TNT [22], respectively. For maximum likelihood, rapid bootstrapping in conjunction with the autoMRE boot stopping criterion [23] and subsequent search for the best tree was used, while for maximum parsimony, 1,000 bootstrapping replicates were used.

The phylogenetic tree showed the affiliations of strain PWU4^T, PWU5^T, PWU20^T and PWU37^T belonging to the family *Cytophagaceae* but also showed that there are no close relatives described (Figure 1) and they shared in range of 91.3 up to 97.5 % 16S rRNA gene sequence similarity within each other. On the report of the NCBI database, the closest relatives of strain PWU4^T were *Ohtaekwangia koreensis* 3B-2^T (92.1 % 16S rRNA gene sequence similarity), *Ohtaekwangia kribbensis* 10AO^T (92.0 %), *Chryseolinea soli* KIS68-18^T (91.0 %), whereas the closest relatives of strain PWU5^T were *Ohtaekwangia koreensis* 3B-2^T (93.6 % 16S rRNA gene sequence similarity), *Ohtaekwangia kribbensis* 10AO^T (93.1 %), *Chryseolinea serpens* RYG^T (92.3 %). The closest relatives of strain PWU20^T were *Ohtaekwangia kribbensis* 10AO^T (92.5 % 16S rRNA gene sequence similarity), *Ohtaekwangia koreensis* 3B-2^T (92.0 %), *Chryseolinea soli* KIS68-18^T (90.6 %). Moreover, the closest relatives of strain PWU37^T were *Ohtaekwangia kribbensis* 10AO^T (93.7 % 16S rRNA gene sequence similarity), *Ohtaekwangia koreensis* 3B-2^T (93.2 %), *Chryseolinea soli* KIS68-18^T (92.0 %).

Genome Features

Draft genome sequences of four strains were obtained as described previously [24]. The DNA G+C content of strain PWU4^T; PWU5^T; PWU20 ^{T}; and PWU37^T were 50.2 mol %; 51.6 mol %; 39.8 mol % and 53.8 mol %, respectively. Further, the differences in G+C content were more than 2.3 % and thus supporting distinct

species [25]. The pairwise digital DNA-DNA hybridization (dDDH) revealed value of 13 % to 50 % and confirmed that all of strains represent new species.

The average nucleotide identity (ANI) values between the genome of the strains and their closest relatives were calculated with the OrthoANIu algorithm using the EZ-Genome web service [26]. Strain PWU4^T; PWU5^T; PWU20 ^{T}; and PWU37^T shared ANI value of 69.2 %; 69.9 %; 69.5 % and 69.7 % with *Ohtaekwangia koreensis* 3B-2^T and value of 68.2 %; 67.6 %; 68.7 % and 67.6 % with *Chryseotalea sanaruensis* Ys^T, respectively. The low ANI value below the threshold 95.0 %-96.0 % [27] confirming that all of strains represent different species for each other.

Physiology and Chemotaxonomy

Phenotypic characterization was performed following to protocols described previously [6, 7, 28]. Morphological characteristic of the strain was observed using the light microscope (Zeiss Axio Scope A1. Microscope) with Axio-Vision Rel. 4.8 software. The cells of strain PWU4^T, PWU5^T, PWU20 ^{T} and PWU37^T were straight rods, 2.32-7.62 µm in length, stained Gram-negative and form yellow colonies on E medium [29].

Growth at various temperature, pH and NaCl concentrations was carried out aerobically on E agar medium. To determine the optimal temperature and pH, duplicate plates were incubated at 4-44^{o}C and also at pH 5.0-9.5 as described previously [24]. Strain PWU4^T and PWU20^T grew at 21-40^{o}C (optimum at 28-30^{o}C), while strain PWU5^T and PWU37^T grew at 21-34^{o}C (optimum at 28-34^{o}C). Moreover, strain PWU4^T grew at pH 5.5-8.0 (optimum at pH 7), strain PWU5^T grew at pH 6.5-8.5 (optimum at pH 7), strain PWU20^T grew at pH 6.5-9.0 (optimum at pH 7) while strain PWU37^T grew at pH 5.0-9.5 (optimum at pH 7.4-8.0). In contrast to previous study, reference strains [28] optimally grew at 30^{o}C in range 10-39^{o}C and pH 6.5-7.5 (Table 1). Salt tolerance was performed in range 0.2-1.6 % NaCl (w/v). Strain PWU4^T, PWU5^T, PWU20 ^{T} and PWU37^T could tolerate a concentration up to 0.4, 0.6, 0.8 and 1.0 % (w/v), respectively. However, within in this study reference strains such as members of the genus *Ohtaekwangia,* which were isolated from marine environment, tolerate a concentration up to 0.2 % (w/v). Other of the closest genera such as *Chryseotaela* tolerate up to 1.0 % of NaCl (w/v) [7] and *Chryseolinea* up to 0.1 % of NaCl (w/v) [6].

Anaerobic growth was performed using E agar plates with Anaerocult P (Merck) in a candle jar [30] among 3 weeks of incubation. No growth was observed for all the strains under anaerobic conditions. Reichenbach [17] noted that a few of the member of *Cytophagaceae* grow microaerophilic, capnophilic (CO_2-requiring) or facultative anaerobic.

Catalase and oxidase activities were performed following Yoon *et al*. [7] and the production of flexirubin-type pigments was tested following Reichenbach [17]. Catalase and oxidase activities along with flexirubin-type pigments for all the strain were negative and also for the reference strains (Table 1).

Carbon sources utilization assay was carried out in duplicate using E broth medium along with the Gen III MicroPlate system (Biolog) following to the manufacturer's protocol. Strain PWU4^T, PWU5 ^{T}, PWU20 ^{T} and PWU37 ^{T} were able

to metabolize D-galactose and glutamic acid and not able to use dextrin and N-acetyl-D-glucosamine (Table 1).

Enzyme activities was performed using the API ZYM [31] and API CAMPY [32] system (bioMérieux), according to the protocols of manufacturer. Strain PWU4^T, PWU5 ^{T}, PWU20 ^{T} and PWU37 ^{T} along with reference strains (Table 1) were positive for phosphatase alkaline, leucine arylamidase, N-Acetyl-β-glucosamidase, L-arginine arylamidase, L-aspartame arylamidase and alkaline phosphatase. Negative test was obtained for pyrrolidinyl arylamidase, reduce nitrate and H_2S production.

Antibiotic resistances was tested on E agar medium following disc-diffusion plate method [33]. Strain PWU4^T, PWU5 ^{T}, PWU20 ^{T} and PWU37 ^{T} were resistant to Polymyxin [50µg/mL], Kanamycin [50µg/mL], Ampicillin [100 µg/mL] and vice versa to Chloramphenicol [30µg/mL].

For chemotaxonomic analysis strain PWU4^T, PWU5 ^{T}, PWU20 ^{T} and PWU37 ^{T} were grown on M broth medium. The Freeze-dried cells were prepared for detection of the major isoprenoid quinone, the polar lipid and the cellular fatty acid of the strains. In brief, 200 ml well-grown cultures were centrifuged at 9,000 rpm for 10 min and the pellet was washed three times following centrifugation 9,000 rpm for 10 min and continued by freeze-drying for two days. The isoprenoid quinone was tested following Komagata and Suzuki [34] and analyzed further using HPLC (Agilent 1260 Series; Agilent technology USA). The polar lipid was determined by two-dimensional TLC [35, 36].

Furthermore, the Sherlock Microbial Identification System (MIDI) as used for identifying cellular fatty acids [37]. The major respiratory quinone is menaquinone MK-7 for all the strain. The menaquinone MK-7 was also found in closest genera (Table 1) and almost the member of the phylum *Bacteroidetes* [6, 7, 38] except the family *Flavobacteriaceae*, which have menaquinones of type 6 (MK6) [2]. The major polar lipids of strain PWU4^T, PWU5^T, PWU20^T and PWU37^T were phosphatidylethanolamine (PE) and another unknown polar lipid. The results of fatty acids were shown in Table 3, along those reference strains obtained in this study. Saturated and monounsaturated fatty acids with iso $c_{15:0}$ and $c_{16:1}$ ω7c were performed in all the strain including reference strains. The major fatty acid of strain PWU4^T, PWU5^T, PWU20^T and PWU37^T were $c_{16:1}$ ω7c (32.5 %), $c_{16:1}$ ω5c (43.8 %), iso $c_{15:0}$ (43.6 %) and $c_{16:1}$ ω5c (38.5 %), respectively. In contrast to strain PWU4^T where $c_{16:1}$ ω7c was found to be the major fatty acid but was much less abundant in strain PWU37^T (9.0 %). Moreover, $c_{16:1}$ ω5c as the major fatty acids in strain PWU5^{T}and PWU37^T but vice versa to strain PWU4^T and PWU20^T. McBride *et al*. [2] highlighted that branched, unsaturated or hydroxyl fatty acids represent the predominant cellular fatty acids in most genera.

Morphological, biochemical, physiological and phylogenetically characteristic of strains PWU4^T, PWU5 ^{T}, PWU20 ^{T} and PWU37 ^{T} confirmed the position within the family *Cytophagaceae.* However, the obtained genetic data between strains PWU4^T, PWU5^T, PWU20^T, PWU37^T and related genera were more important to differentiate them from known genera of the family *Cytophagaceae.* Therefore, strains PWU4^T, PWU5^T, PWU20^T and PWU37^T should be classified in two new genera within the family *Cytophagaceae.* Furthermore, a phylogenetic tree based on genomic data of all the strains revealed that strain PWU4^T and PWU20 ^{T} form together one genus with different species (the low ANI value 69.73 %) and

the other strains PWU37 ^{T} and PWU5 ^{T} also form together one genus with different species (the low ANI value 83.85 %).

Protologue

DESCRIPTION OF *CRYSEOSOLUM* GEN. NOV.

Cryseosolum (Cry.se.o.so`lum. Gr. adj. *chryseos*, golden; *solum*, soil; N.L. *Cryseosolum* s yellow-colored bacteria from soil).

Gram-stain-negative, non-sporulating, non-motile, mesophilic, heterophilic and aerobic bacteria. Cells are rod-shaped and catalase- as well as oxidase-negative. Growth is observed on D-gluconic acid and methyl pyruvate. The major cellular fatty acids are *iso*-$C_{15:0}$ and $C_{16:1}$ w7c. The major respiratory quinone is menaquinone-7 (MK-7). The major identified polar lipid was phosphatidylethanolamine (PE). Member of the class *Cytophagia*, order *Cytophagales*, phylum *Bacteriodetes.* The type species is *Cryseosolum histidinii.*

DESCRIPTION OF *CRYSEOSOLUM HISTIDINII* SP. NOV.

Cryseosolum histidinii (his.ti.din`ii. N.L. mask. adj. *histidinii* pertaining to histidine, an amino acid, which can be utilized by this strain).

In addition to the characteristic listed in the genus description, the species has the following characteristics: Cells are 2.56-6.67 µm long and appear as single cells. Colonies are irregular and yellow on E agar plate after 5 days of cultivation. Growth occures between 21 and 40 ºC (optimum 30 ºC), between pH 5.5 and 8.0 (optimum pH 7) and tolerance with occur below 0.4 % (w/v) of NaCl. Positive for phosphatase alkaline, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, phosphatase acid, naphthol-AS-B1-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosamidase, α-mannosidase, α-fucosidase, urease, esterase and alcaline phosphatase. Weakly activities on lipase (C14) and β-glucoronidase. No growth is observed on dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, α-D-lactose, D-melibiose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, α-D-glucose, D-mannose, L-fucose, D-arabitol, glycerol, gelatin, glycyl-L-proline, L-alanine, L-arginine, L-serine, pectin, D-galacturonic Acid, L-galactonic acid lactone D-gluconic acid, glucuronic acid, glucuornicamide, mucic acid, D-lactic acid methyl ester, citric acid, D-malic acid, L-malic acid, bromo-succinic acid, tween 40, γ-amino-butryric acid, α-keto-butyric-acid, acetoaacetic acid, and acetic acid.

The type strain is PWU4^{T} (=DSM 111594^{T} = NCCB 100798^{T}) isolated from a soil samples collected in May 1990 at Braunschweig, Germany (52.22090 N 10.50902 E). The DNA G+C content of the type strain is 50,2 mol %. The 16S rRNA gene and whole-genome sequences have been deposited in GenBank/EMBL/DDBJ under accession numbers MW182516 and ERR4837147, respectively.

DESCRIPTION OF *CRYSEOSOLUM INDIENSIS* SP. NOV.

Cryseosolum indiensis (in.di.en. `sis. N.L. fem. adj. *indiensis* referring to India from which soil was isolated).

In addition to the characteristic listed in the genus description, the species has the following characteristics: Cells are 2.32-6.41 µm long and appear as single cells. Colonies are irregular and yellow on E agar plate after 5 days of cultivation. Growth occures between 21 and 40 ºC (optimum 28 ºC), between pH 6.5 and 9.0 (optimum pH 7) and tolerance with occur below 0.8 % (w/v) of NaCl. Positive for phosphatase alkaline, leucine arylamidase, valine arylamidase, cystine arylamidase, chymotrypsin, phosphatase acid, naphthol-AS-B1-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucoronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosamidase, α-mannosidase, urease, esterase and alcaline phosphatase. Weakly activities on esterase (C4), esterase lipase (C8), lipase (C14), trypsin and no activities for α-fucosidase. No growth is observed on dextrin, D-maltose, D-cellobiose, gentiobiose, N-acetyl-D-glucosamine, N-acetyl-β-D-galactosamine, D-fructose, D-frucose, L-rhamnose, insosine, D-sorbtiol, D-mannitol, D-arabitol, myo-inositol, D-fructose-6-PO4, L-alanine, L-arginine, L-histidine, L-galactonic acid lactone D-gluconic acid, p-hydroxy-phenylacetic acid, L-malic acid, bromo-succinic acid, tween 40, α-keto-butyric-acid, propionic acid, and acetic acid.

The type strain is PWU20^{T} (=DSM 111595^{T} = NCCB 100800^{T}) isolated from a soil samples collected in May 1989 at Lucknow, Uttar Pradesh, India (26.8684 N 80.90979 E). The DNA G+C content of the type strain is 39,8 mol %. The 16S rRNA gene and whole-genome sequences have been deposited in GenBank/EMBL/DDBJ under accession numbers MW182517 and ERR4837148, respectively.

DESCRIPTION OF *REICHENBACHIA* GEN. NOV.

Reichenbachia (rei.chen.bach`i.a. N.L. fem. Adj. *reichenbachia* referring to Hans Reichenbach, who was a famous Germany microbiologist in research on gliding bacteria).

Gram-stain-negative, non-sporulating, non-motile, mesophilic, heterophilic and aerobic bacteria. Cells are form rod-shaped and catalase- as well as oxidase-negative. Growth observed on D-cellobiose, L-fucose, D-arabitol, L-malic acid, and α-keto-butyric-acid. The major cellular fatty acids are *iso*-$C_{15:0}$ and $C_{16:1}$ w5c. The major respiratory quinone is menaquinone-7 (MK-7). The major identified polar lipids were phosphatidylethanolamine (PE). Of the class *Cytophagia*, order *Cytophagales*, phylum *Bacteriodetes.* The type species is *Reichenbachia cretensis.*

DESCRIPTION OF *REICHENBACHIA CRETENSIS* SP. NOV.

Reichenbachia cretensis (cre.ten.`sis. N.L. fem. adj. *cretensis* referring to Crete, from which soil was isolated).

In addition to the characteristic listed in the genus description, the species has the following characteristics: Cell are 4.37-7.62 µm long and appear as single cells. Colonies are irregular and yellow on E agar plate after 5 days of cultivation. Grow occur between 21 and 34 ºC (optimum 28 ºC), between pH 6.5 and 8.5 (optimum

pH 7) and tolerance with occur below 0.6 % (w/v) of NaCl. Positive for phosphatase alkaline, leucine arylamidase, valine arylamidase, cystine arylamidase, phosphatase acid, naphthol-AS-B1-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosamidase, esterase and alcaline phosphatase. Weakly activities on esterase (C4), esterase lipase (C8), lipase (C14) and no activities for trypsin, chymotrypsin, β-glucoronidase, α-mannosidase, α-fucosidase and urease. No growth is observed on dextrin, D-turanose, α-D-lactose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, L-arginine, L-histidine, L-pyroglutamic acid, D-gluconic Acid, glucuronic acid, glucuornicamide, mucic acid, quinic acid, D-saccharic acid, methyl pyruvate, citric acid, γ-amino-butryric acid, and β-hydroxy-D,L-butyric acid.

The type strain is PWU5^T (=DSM 11596^T = NCCB 100799^T) isolated from faces sheep with plant residues collected in July 1988 at Crete Island (35.2463 N 25.09705 E). The DNA G+C content of the type strain is 51,6 mol %. The 16S rRNA gene and whole-genome sequences have been deposited in GenBank/EMBL/DDBJ under accession numbers MW182518 and ERR4837149, respectively.

DESCRIPTION OF *REICHENBACHIA SOLI* SP. NOV.

Reichenbachia soli (so.li. N.L. mask. Adj. soli referring to soil, where it was isolated from Braunschweig, Germany).

In addition to the characteristic listed in the genus description, the species has the following characteristics: Cell are 3.1-6.43 μm long and appear as single cells. Colonies are irregular and yellow on E agar plate after 5 days of cultivation. Growth occures between 21 and 34 ºC (optimum 34 ºC), between pH 5.0 and 9.5 (optimum pH 7.4 until 8.0) and tolerance with occur below 1.0 % (w/v) of NaCl. Positive for phosphatase alkaline, leucine arylamidase, valine arylamidase, cystine arylamidase, phosphatase acid, naphthol-AS-B1-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosamidase, α-fucosidase, esterase and alcaline phosphatase. Weakly activities for of esterase (C4), esterase lipase (C8), lipase (C14) and no activities for trypsin, chymotrypsin, β-glucoronidase, α-mannosidase, and urease. No growth is observed on dextrin, D-maltose, gentiobiose, D-turanose, D-raffinose, α-D-lactose, D-melibiose, D-salicin, N-acetyl-D-glucosamine, N-acetyl-β-D-galactosamine, N-acetyl-D-galactosamine, α-D-glucose, D-frucose, D-sorbtiol, myo-inositol, D-glucose-6-PO4, D-fructose-6-PO4, D-aspartic acid, D-serine, gelatin, L-alanine, L-arginine, L-aspartic Acid, L-histidine, pectin, L-galactonic acid lactone D-gluconic acid, D-gluconic acid, glucuornicamide, mucic acid, p-hydroxy-phenylacetic acid, methyl pyruvate, D-lactic acid methyl ester, L-lactic acid, citric acid, α-keto-glutaric acid, D-malic acid, bromo-succinic acid, tween 40, α-hyydroxy-butyric acid, β-hydroxy-D,L-butyric acid, acetoaacetic acid, propionic acid, acetic acid and formic acid.

The type strain is PWU37^T (=DSM 111597^T = NCCB 100801^T) isolated from a soil samples collected in September 1991 at Braunschweig (52.21501 N 10.53329 E). The DNA G+C content of the type strain is 53,8 mol %. The 16S rRNA gene and whole-genome sequences have been deposited in GenBank/EMBL/DDBJ under accession numbers MW182519 and ERR4837150, respectively.

AUTHOR STATEMENTS

Authors and contributors

Conceptualized: SO, JW. Supervised: JW, UN. Performed the experiments except genome: SO, SL, FA -genome experiment: UN. Analyzed the data: SO, SL, FA. Wrote the paper-original draft preparation: SO, SL. –review and edited SO, SL, UN and JW.

Conflicts of interest

The author declares that there are no conflicts of interest

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Ethical approval

This research did not contain any studies with human or animals performed by any of the authors.

Consent for publication

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ABBREVIATIONS

DSMZ	*Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH*
GGDC	Genome to Genome Distance Calculator
G+C	Guanine + Cytosine
MEGA X	Molecular Evolutionary Genetics Analysis
NCBI	National Center for Biotechnology Information
PAUP*	Phylogenetic Analysis Using Parsimony
PCR	Polymerase Chain Reaction
RaxML	Randomized Accelerated Maximum Likelihood
RDP	Ribosomal Database Project
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
TNT	Tree analysis using New Technology

REFERENCES

1. **Stanier RY**. Studies on the Cytophagas. *J Bacteriol* 1940;40:619–635.
2. **Albers SV, Siebers B**. *The family sulfolobaceae*. 2014. Epub ahead of print 2014. DOI: 10.1007/978-3-642-38954-2_329.
3. **Nakagawa Y**. Family I. Cytophagaceae Stanier 1940, 630AL. *Bergey's Man Syst Bacteriol* 2011;4:371.
4. **Hirsch P, Ludwig W, Hethke C, Sittig M, Hoffmann B, *et al.*** Hymenobacter roseosalivarius gen. nov., sp. nov. from continental Antartica soils and sandstone: bacteria of the Cytophaga/Flavobacterium/Bacteroides line of phylogenetic descent. *Syst Appl Microbiol* 1998;21:374–383.
5. **Zhang J-Y, Liu X-Y, Liu S-J**. Adhaeribacter terreus sp. nov., isolated from forest soil. *Int J Syst Evol Microbiol* 2009;59:1595–1598.
6. **Kim J-J, Alkawally M, Brady AL, Rijpstra WIC, Sinninghe Damsté JS, *et al.*** Chryseolinea serpens gen. nov., sp. nov., a member of the phylum Bacteroidetes isolated from soil. *Int J Syst Evol Microbiol* 2013;63:654–660.
7. **Maejima Y, Iino T, Muraguchi Y, Fukuda K, Ohkuma M, *et al.*** Chryseotalea sanaruensis gen. nov., sp., nov., a Member of the Family Cytophagaceae, Isolated from a Brackish Lake in Hamamatsu Japan. *Curr Microbiol* 2020;77:306–312.
8. **Baik KS, Seong CN, Moon EY, Park Y-D, Yi H, *et al.*** Hymenobacter rigui sp. nov., isolated from wetland freshwater. *Int J Syst Evol Microbiol* 2006;56:2189–2192.
9. **Buczolits S, Denner EBM, Vybiral D, Wieser M, Kämpfer P, *et al.*** Classification of three airborne bacteria and proposal of Hymenobacter aerophilus sp. nov. *Int J Syst Evol Microbiol* 2002;52:445–456.
10. **Zhou Y, Wang X, Liu H, Zhang K-Y, Zhang Y-Q, *et al.*** Pontibacter akesuensis sp. nov., isolated from a desert soil in China. *Int J Syst Evol Microbiol* 2007;57:321–325.
11. **Chaturvedi P, Reddy GSN, Shivaji S**. Dyadobacter hamtensis sp. nov., from Hamta glacier, located in the Himalayas, India. *Int J Syst Evol Microbiol* 2005;55:2113–2117.
12. **Katayama N, Nozaki Y, Okonogi K, Ono H, Harada S, *et al.*** Formadicins, new monocylic b-lactam antibiotics of bacterial origin. *J Antibiot* 1985;39:1117–1127.
13. **Ikegami Y, Takeuchi N, Hanada M, Hasegawa Y, Ishii K, *et al.*** Topostin, a novel inhibitor of mammalian DNA topoisomerase I from Flexibacter topostinus sp. nov. II. Purification and some properties of topostin. *J Antibiot (Tokyo)* 1990;43:158–162.
14. **Singh PD, Ward PC, Scott Wells J, Ricca CM, Trejo WH, *et al.*** Bacterial Production of Deacetoxycephalosporin C. *J Antibiot (Tokyo)* 1982;35:1397–1399.
15. **Park JH, Kim R, Aslam Z, Jeon CO, Chung YR**. Lysobacter capsici sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus Lysobacter. *Int J Syst Evol Microbiol*

2008;58:387–392.
16. **Okanya PW, Mohr KI, Gerth K, Jansen R, Müller R**. Marinoquinolines A-F, pyrroloquinolines from ohtaekwangia kribbensis (Bacteroidetes). *J Nat Prod* 2011;74:603–608.
17. **Reichenbach H**. *The Order Cytophagales*. 1992. Epub ahead of print 1992. DOI: 10.1007/978-1-4757-2191-1_37.
18. **Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M**. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
19. **Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M**. Highly parallelized inference of large genome-based phylogenies. *Concurr Comput Pract Exp* 2014;26:1715–1729.
20. **Edgar RC**. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792–1797.
21. **Stamatakis A**. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
22. **Goloboff PA, Farris JS, Nixon KC**. TNT, a free program for phylogenetic analysis. *Cladistics* 2008;24:774–786.
23. **Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A**. How many bootstrap replicates are necessary? *J Comput Biol* 2010;17:337–354.
24. **Mohr KI, Wolf C, Nübel U, Szafrańska AK, Steglich M, *et al.*** A polyphasic approach leads to seven new species of the cellulose-decomposing genus Sorangium, Sorangium ambruticinum sp. nov., Sorangium arenae sp. nov., Sorangium bulgaricum sp. nov., Sorangium dawidii sp. nov., Sorangium kenyense sp. nov., Sorangium or. *Int J Syst Evol Microbiol* 2018;68:3576–3586.
25. **Meier-Kolthoff JP, Klenk H-P, Göker M**. Taxonomic use of DNA G+C content and DNA–DNA hybridization in the genomic age. *Int J Syst Evol Microbiol* 2014;64:352–356.
26. **Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, *et al.*** Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
27. **Richter M, Rosselló-Móra R**. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* 2009;106:19126–19131.
28. **Yoon JH, Kang SJ, Lee SY, Lee JS, Park S**. Ohtaekwangia koreensis gen. nov., sp. nov. and Ohtaekwangia kribbensis sp. nov., isolated from marine sand, deep-branching members of the phylum Bacteroidetes. *Int J Syst Evol Microbiol* 2011;61:1066–1072.
29. **Okanya PW, Mohr KI, Gerth K, Jansen R, Müller R**. Isolation and structure elucidation of secondary metabolites from the gliding bacteria Ohtaekwangia kribbensis and Hyalangium minutum. *J Nat Prod* 2011;74:603–608.
30. **Jones DM**. Manual of Methods for General Bacteriology. *Journal of Clinical Pathology* 1981;34:1069.
31. **Humble MW, King A, Phillips I**. API ZYM: a simple rapid system for the

detection of bacterial enzymes. *J Clin Pathol* 1977;30:275–277.
32. **Huysmans MB, Turnidge JD, Williams JH**. Evaluation of API Campy in comparison with conventional methods for identification of thermophilic campylobacters. *J Clin Microbiol* 1995;33:3345–3346.
33. **Bauer AW, Kirby WM, Sherris JC, Turck M**. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966;45:493–496.
34. **Komagata K, Suzuki K-I**. 4 Lipid and Cell-Wall Analysis in Bacterial Systematics. In: Colwell RR, Grigorova R (editors). *Current Methods for Classification and Identification of Microorganisms*. Academic Press. pp. 161–207.
35. **Lechevalier MP, De Bievre C, Lechevalier H**. Chemotaxonomy of aerobic Actinomycetes: Phospholipid composition. *Biochem Syst Ecol* 1977;5:249–260.
36. **Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, *et al.*** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.
37. **Sasser M**. Technical Note # 101 M icrobial Identification by Gas Chromatograph ic Analysis of Fatty Acid Methyl Esters (GC-FAME). *Tech Note* 2009;1–6.
38. **Yoon JH, Kang SJ, Lee SY, Lee JS, Park S**. Ohtaekwangia koreensis gen. nov., sp. nov. and Ohtaekwangia kribbensis sp. nov., isolated from marine sand, deep-branching members of the phylum Bacteroidetes. *Int J Syst Evol Microbiol* 2011;61:1066–1072.

FIGURES AND TABLES

Figure 1. Neighbor-joining phylogenetic tree of partial 16S rRNA gene sequences of strain PWU4^T, PWU5^T, PWU20^T and PWU37^T with other representatives of the phylum Bacteriodetes. Bootstrap value (1000 resampling) at branch nodes (Maximum Parsimony/Maximum-Likelihood). Bar 0.09 substitutions per nucleotide position.

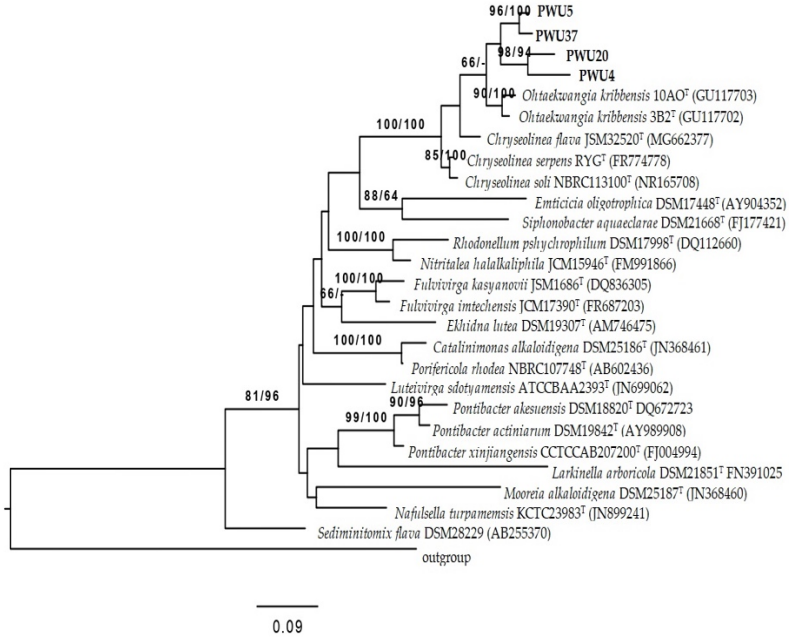

Table 1. Major phenotypic distinguishing strain PWU4^T, PWU5^T, PWU20^T and PWU37^T with closest genera.

Strain: 1, PWU4^T; 2, PWU5^T; 3, PWU20^T; 4, PWU37^T; 5, *Ohtaekwangia koreensis* 3B-2^T and 6, *Ohtaekwangia kribbensis* 10AO^T. +, positive; w. weakly activities, - negative, * taken from Yoon *et al*. [38]

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**
Cell morphology	Rod	Rod	Rod	Rod	Rod	Rod
Cell length [µm]	2.56-6.67	4.37-7.62	2.32-6.41	3.1-6.43	1.0-5.0	1.5-7.5
Temperature range of growth [°C]	21-40	21-34	21-40	21-34	10-39	10-39
Optimal temperature [°C]	30	28	28	34	30	30
pH range of growth	5.5-8.0	6.5-8.5	6.5-9.0	5.0-9.5	5.5-9.0	4.5-9.0
Optimal pH	7	7	7	7.4-8.0	6.5-7.5	6.5-7.5
NaCl tolerance [%NaCl, w/v]	0-0.4	0-0.6	0-0.8	0-1.0	0-0.2	0-0.2
Flexirubin type pigment	-	-	-	-	+	+
Catalase	-	-	-	-	+	+
Oxidase	-	-	-	-	+	+
Enzyme activity (Api®ZYM, Api®CAMPI) :						
Esterase (C4)	+	W	w	w	-	-
Esterase lipase (C8)	+	W	w	w	-	-

Lipase (C14)	w	w	w	w	-	-
Valine arylamidase	+	+	+	+	+	-
Cystine arylamidase	+	+	+	+	-	-
Trypsin	+	-	w	-	+	-
Chymotrypsin	+	-	+	-	-	-
Phosphatase acid	+	+	+	+	+	-
Naphthol-AS-B1-Phosphohydrolase	+	+	+	+	+	-
α-galactosidase	+	+	+	+	+	-
β-galactosidase	+	+	+	+	+	-
β-glucoronidase	W	-	+	-	-	-
α-glucosidase	+	+	+	+	+	-
β-glucosidase	+	+	+	+	+	-
α-mannosidase	+	-	+	-	+	-
α-fucosidase	+	-	-	+	+	-
Urease	+	-	+	-	-	-
Hippurate	+	-	+	-	+	+
γ-glutamyl transferase	+	+	+	+	-	-
Reduction of tetrazolium	+	+	+	+	-	-
Antibiotic resistance:						
Gentamycin [50 μg/ml]	+	+	-	+	-	+
G+C contents [mol%]	50,2	51,6	39,8	53,8	42,8*	44,6*
Carbon sources utilization:						
D-Cellobiose	-	+	-	+	+	+
L-Fucose	-	+	-	+	+	+
D-Arabitol	-	+	-	+	-	-
D-Gluconic Acid	+	-	+	-	+	+
Methyl Pyruvate	+	-	+	-	-	-
L-Malic Acid	-	+	-	+	-	+
α-Keto-Butyric-Acid	-	+	-	+	-	-

Table 2. Cellular fatty acid composition of strain PWU4^T, PWU5^T, PWU20^T and PWU37^T with closest genera.

Strain: 1, PWU4^T; 2, PWU5^T; 3, PWU20^T; 4, PWU37^T; 5, *Ohtaekwangia koreensis* 3B-2^T and 6, *Ohtaekwangia kribbensis* 10AO^T. +, positive; - negative. Values are percentages of total fatty acids. -, not detected, ECL, equivalent chain-length.

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**
Straight-chain						
$c_{14:0}$	1,9	-	1,3	1,0	-	-
$c_{15:0}$	1,8	2,3	-	1,6	-	2,1
$c_{16:0}$	-	2,8	9,7	9,2	-	22,2
$c_{17:0}$	-	1,3	-	-	-	-
$c_{18:0}$	2,7	-	1,1	-	-	-
Branched						
iso $c_{13:0}$	-	-	-	-	-	-
iso $c_{14:0}$	-	-	-	-	-	1,7
iso $c_{15:0}$	22,0	26,9	43,6	38,2	20,4	30,2
iso $c_{16:0}$	12,9	2,2	1,4	-	9,5	4,0
iso $c_{17:0}$	-	-	1,2	1,0	-	7,4
Unsaturated						
$c_{15:1}$ ω7c	1,4	2,8	0,8	-	-	-
$c_{16:1}$ ω5c	-	43,8	-	38,5	-	-
$c_{16:1}$ ω7c	32,5	16,6	32,0	9,0	55,2	27,4
$c_{16:1}$ ω8c	-	-	-	0,7	-	-
$c_{17:1}$ ω7c	-	-	1,0	-	-	-
$c_{18:1}$ ω9c	8,9	-	3,4	-	-	-
$c_{18:2}$ ω6,9c	13,6	-	4,5	-	-	-
Hydroxy						
$C_{14:0}$ 2-OH	1,0	-	-	-	-	-
$C_{16:0}$ 2-OH	1,3	-	-	-	-	-
Unknown						
ECL 11.864	-	-	-	-	6,2	3,3
ECL 12.558	-	1,2	-	-	-	-
ECL 16.089	-	-	-	0,7	-	-
ECL 22.207	-	-	-	-	8,7	1,7

Supplementary 6

ORIGINAL RESEARCH

Myxobacteria Isolated from Indonesian Mangroves as Sources of Novel Bioactive Compound

Senlie Octaviana [1, 2, *], Gian Primahana [3, 4], Tjandrawati Mozef [4], Luiz G. A. Borges [5], Dietmar H. Pieper [5], and Joachim Wink [1, *]

[1] Microbial Strain Collection, Helmholtz Center for Infection Research, Braunschweig, Germany
[2] Research Center for Biotechnology LIPI, Cibinong, Jawa Barat, Indonesia
[3] Microbial Drug, Helmholtz Center for Infection Research, Braunschweig, Germany
[4] Research Center for Chemistry LIPI, Tangerang Selatan, Indonesia
[5] Microbial Interactions and Processes, Helmholtz Center for Infection Research, Braunschweig, Germany

* Correspondence
Senlie Octaviana, Email : Senlie.Octaviana@helmholtz-hzi.de
Joachim Wink, Email : Joachim.Wink@helmholtz-hzi.de

Abstract
Mangroves are unique intertidal ecosystems that provide ecological niches to different microbes, which play various roles in nutrient recycling and diverse environmental activities. The association between myxobacteria and mangroves are hitherto poorly understood. The aim of our study was to evaluate the myxobacterial community composition and to characterize the antimicrobial activity of myxobacteria isolates from Indonesian mangroves. Twenty-five cultivable myxobacteria were affiliated in six genera: *Myxococcus*, *Corallococcus*, *Archangium*, *Chondromyces*, *Racemicystis* and *Nannocystis* of the order *Myxococcales* based on partial 16S rRNA gene sequences. Thirteen crude extracts showed moderate activities against at least one of human pathogenic microorganisms. The crude extract of *Racemicystis* sp. strain 503MSO indicated a novel compound, which has not been reported in the database yet and the identification of this compound needs further study. The myxobacterial communities of three different sampling sites were analyzed using primers adapted for the myxobacteria group identification. The results showed that myxobacterial communities are more diverse than assumed. Therefore, our study has highlighted the importance of the mangrove habitat as promising harbor of myxobacteria as well as novel antimicrobial compounds with activity against pathogenic microorganisms.
Keywords : mangrove, myxobacteria, antimicrobial, bioactive compound

1. INTRODUCTION

One of the most fascinating groups producing natural microbial products are myxobacteria [1,2]. There are more than a hundred new carbon skeletons and derivatives of compounds with antibacterial, antifungal, antimalarial, antioxidative,

or antiviral activity [3–6]. However, discovering new bioactive compounds from nature is still required, due to the increasing antimicrobial resistance.

Myxobacteria are Gram-negative *Deltaproteobacteria* possessing a social lifestyle with moving, preying, and surviving cooperatively in predatory groups [7]. Their communities are commonly found in various environments, even in extreme conditions [7–9]. A preliminary study considered the use of specific primers and probes to analyze myxobacterial diversity in soil samples [10] in addition Li *et al.* [11] and Brinkhoff *et al.* [12] carried out PCR-DGGE (Denaturing Gradient Gel Electrophoresis) and quantitative real time PCR to define myxobacteria diversity. Besides, clone bank analyses, cultivation approaches were also used to compare their diversity from different habitats [13].

Mangroves are unique environments of small forest trees in brackish water with transitional zones in the coastal area. They tolerate a wide range of salinity, oxygen, nutrient and harbor many types of microorganisms [14], [15]. Antimicrobial compounds of different microbial origin have been documented from mangroves [16,17]. However, there is limited information about the antimicrobial potential of myxobacteria isolated from Indonesian mangroves, which are known as the world's largest mangroves [18].

Presently, next generation sequencing (NGS) of hypervariable regions of the 16S rRNA gene, is a tool that gives profound insights into microbial communities. Jiang *et al.* [14] stated tremendous success using this method in comparing the bacterial community diversity and composition between mudflat, edge, bulk, and rhizosphere of mangrove's samples in Hong Kong, China. Furthermore, Linares-Otoya *et al*. [19] used NGS to report predatory bacteria from the Peruvian coastline stating that the microbiome present in this region is a promising source for heterotrophic bacterial strains and has potential for bioprospecting of antibiotics.

The use of culture-independent methods is of great interest, as it has identified a larger number of novel taxa when compared to culture-dependent methods under standard laboratory condition [13,14,19,20]. However, for further analysis, the novel taxa need to be isolated from nature. This procedure depends on culture methods and is crucial for the production of secondary metabolites in fermentation processes [21].

Ambruticin was the first antimicrobial metabolite isolated from a myxobacterium, *Sorangium* sp. [21]. Mohr highlighted that most of the myxobacterial secondary metabolites are polyketides, non-ribosomal polypeptides and derivatives thereof and many strains of the suborder *Sorangiineae* produce these compounds [21]. Ultimately, there are higher chances of discovering novel antimicrobial compounds from novel myxobacteria compared to other genera of members of the order *Myxococcales* [22].

The aim of this study was to isolate myxobacteria, to evaluate the myxobacterial community composition in Indonesian mangroves by Illumina sequencing of 16S rRNA genes using specific primers and to assess their potential for antimicrobial activity.

2. MATERIAL AND METHODS

2.1. Sample Collection

The mangrove samples were collected from Taman Muara Tawar, Bekasi (MT) (6^{o}088772'N 107^{o}002393'E), Taman Muara Angke, Jakarta (MA)

(6º105321'N 106º735578'E), and Taman Mangrove Api-Api, Yogyakarta (MK) (7º894662'S 110º02554'E), at Java, Indonesia in November 2018 (Figure 1). Total of 6 samples including sediment and leaf flakes were taken from each location. Approximately 50 g of the upper sediment with leaf, which fell on it, were placed in a sterile zip lock plastic bag. All samples were air dried at 30ºC for minimalizing contamination from fungi.

2.2. Isolation of Myxobacteria

The strains were isolated as previously described by Mohr *et al*. [13] using water agar with autoclaved *Escherichia coli* strain K12 and Stan 21 agar with filter paper [23]. Swarming colonies or fruiting bodies were observed under dissecting microscope (Olympus SZX10) every five to fifteen days and transferred to new water agar plates with *Escherichia coli* strain K12 and finally to VY/2 agar plates [24]. The pure cultures from VY/2 agar plates were transferred into 20 ml CY/H liquid medium [23] and 1.5 mL of well-grown cultures were directly conserved at -80ºC.

2.3. Identification of Pure Cultures by 16S rRNA Gene Sequences

The bacterial DNA was extracted using Invitek Spin Plant Mini Kit (Invisorb) following the manufacturer's instruction. One microliter of template DNA was directly amplified using F27/R1525 as described [13,20]. The PCR products were checked by 0.8 % agarose gel electrophoresis at 70 V for 40 min and purified by NucleoSpin Gel and PCR Clean up Kit (Macherey-Nagel, Düren, Germany). The forward and reverse sequences of 16S rRNA gene fragments were assembled with the BioEdit program [25] and closely related type strains were identified using the NCBI 16S rRNA gene database. Sequences were deposited at NCBI database under accession number MW199130, MW182265-MW182288.

2.4. Microbiome Analysis

The total DNA was extracted from 250 mg of sample using the MOBio PowerSoil® Kit following the manufacturer's instructions. The quality of DNA was measured in Nano-photometer IMPLAN. Amplification was performed using PrimerSTAR HS DNA Polymerase (Takara, Otsu, Shigu, Japan) following the manufacturer's instructions. Amplification for analyzing the microbial community composition was described by Rath *et al*. [26] with modifications. Forward primers W2 and W5 [10] specifically targeting *Cystobacterineae* and *Sorangiineae*/*Nannocystineae* of the *Myxococcales* were separately used in conjunction with reverse primer R1525 [13,20] in a first PCR reaction. Samples were pre-denaturated at 96°C for 3 min following twenty-five cycles of denaturation at 94°C for 1 min, annealing at 56.6°C for 1 min, extension at 72 °C for 2 min. The PCR product was checked on 2.0 % agarose gel electrophoresis and purified using NucleoSpin Gel PCR Clean up Kit (Macherey-Nagel, Düren, Germany). One microliter of purified PCR product was used as template in a second PCR with primers **807F** and **1050R** containing part of the sequencing primer sites as short overhangs (*ACGACGCTCTTCCGATCT***GGATTAGATACCCBRGTAGTC** and *GACGTCTGCTCTTCCGATCT***AGYTGDCGACRRCCRTGCA**, respectively) for 20 cycles to enrich for target sequences. A third amplification step of 10 cycles

added the two indices and Illumina adapters to amplicons [26]. The second and third PCR reaction following a first step PCR reaction. Obtained products were pooled in equimolar ratios and sequenced on an Illumina Miseq (2x300 bases, San Diego, USA).

The bioinformatic processing was performed as previously described [27]. Raw reads were merged with the Ribosomal Database Project (RDP) assembler [28]. Sequences were aligned within MOTHUR [29] (gotoh algorithm using the SILVA database [30]) and subjected to preclustering (diffs=2) yielding so-called phylotypes that were filtered for an average abundance of ≥ 0.001% and a sequence length ≥ 250 bp before analysis. The potential duplications of same *Myxococcales* sequences between both sets of primers pair were counted and should be included to each primer pair analysis. Phylotypes were assigned to a taxonomic affiliation based on the naive Bayesian classification with a pseudo-bootstrap threshold of 80% [28,31]. All sequences not matching to the order *Myxococcales* were deleted before further analysis. The relative abundance of genera was plotted using pivot table on Microsoft Excel. Sequences were deposited at NCBI database under accession number SRX9502207-SRX9502210 (Project PRJNA678217).

2.5. Preparation for Crude Extracts

Seed cultures from the 25 isolates were prepared from myxobacterial swarming colonies after seven and before fifteen days of incubation (depending on the myxobacteria growth rate) on VY/2 agar medium [23] by inoculation into 20 mL CY/H liquid medium [23]. After seven days, 10% of the seed cultures were transferred into 100 mL screening liquid medium, which contained 2% XAD-absorber resin and incubated at room temperature for seven up to fifteen days. The broth cultures were extracted using 70 mL acetone (J. T. Baker) and the organic phase was evaporated (Heidolph, Laborota 4003 control) at a temperature of 38 to 40°C. The residue was mixed with 1 ml methanol (J. T. baker) and stored in a freezer at -20ºC.

2.6. Screening for Antimicrobial Activity

All 25 crude extracts from 25 strains were characterized by a test panel using the following pathogenic microorganisms: *Escherichia coli* WT-BW 25113, *Escherichia coli* JW0451-02, *Acinetobacteria baumanii* DSM 30008, *Citrobacter freundii* DSM 30039, *Pseudomonas aeruginosa* Pa14, *Staphylococcus aureus* Newmann, *Mycobacterium smegmatis* ATCC 700084, *Candida albicans* DSM 1665, *Wickerhamomyces anomalus* DSM 6766, and *Mucor hiemalis* DSM 2656. The antimicrobial activity was assessed by a growth inhibition test using a serial dilution of each crude extract against different pathogenic microorganisms in a 96-well plate [32]. The highest dilution, at which growth inhibition was observed, was noted. The antimicrobial activity was visualized quantitatively by heat map with Heatmapper software [33].

2.7. Identification of Active Compound

The crude extracts of one active strain (*Racemicystis* sp. strain 503MSO) were fractionated using preparative HPLC (Agilent 1260 Series; Aligent technology USA) for peak-activity correlation [32] as previously described by Okanya *et al.* [34]. The antimicrobial activities of the fractions were analyzed

against those of antimicrobial activity above. Active fractions were further analyzed by HPLC-DAD-UV-HRESIMS as previously described by Primahana *et al.* [35]. The data were analyzed following Krug [36] and Hoffmann [22] using Data Analysis 4.2 B383 (Bruker Daltonics) and identified with the in-House SQL myxobase database.

3. RESULTS

3.1. Mangrove Myxobacteria Isolates

Seventy strains were successfully isolated from three different sources of Indonesian mangroves. All of the strains showed fruiting bodies formation and swarming on a surface agar-medium. Therefore, based on different morphologies and 16S rRNA gene sequences analysis, the number of myxobacteria replicates from same source of organisms and location were reduced and 25 of 70 isolates were obtained. Twenty-five isolates, 16 from MA sampling site, 3 from MT sampling site and 6 from MK sampling site were selected for further analysis. Based on 16S rRNA gene sequence analysis, members of three suborders of *Myxococcales* could be identified (see Table 1). Some isolates showed less than 98.60% similarity to close related type strains. Therefore, full genome sequencing is needed for their further characterization and whether they comprise novel species.

As main characteristics of myxobacteria as previously described by Reichenbach *et al*. [24,37], some of the swarming and fruiting bodies of myxobacterial isolates from mangroves can be seen in Figure 2. *Myxococcus* sp. strains 431MSO and 451MSO have spherical fruiting bodies with yellow or oranges red colors on VY/2 agar medium. *Coralococcus* sp. strain 412MSO makes swarm colonies and forms fruiting bodies with coralloid-branched shapes. *Chondromyces* sp. strain 151MSO builds tree shaped fruiting bodies on Stan21 agar medium and *Archangium* sp. strain 455MSO makes swarm colonies with branched radial veins on VY/2 agar medium. *Racemycistis* sp. strain 503MSO has swarming area like the genus *Sorangium* on VY/2 agar medium.

3.2. Myxobacteria Diversity

Myxobacterial diversity was evaluated after amplification using two different primer pairs W2/R1525 and W5/R1525, targeting the suborders *Cystobacterineae* and *Sorangiineae/Nannocystineae*, respectively [10,13,20]. A total of 20,057 myxobacterial sequences (1761±1002 SEM per sample) from 12 samples, where each sample was amplified twice with the two different primer pairs, were obtained. The success of the W2/R1525 primer to amplify members of the order *Myxococcales* order varied and between 0.56 % and 70.42 % of the obtained reads belonged to this order (see Table 2). With the W5/R1525 primer pair, 7.27-27.03 % of amplified sequences were belonging to *Myxococcales*, indicating a low abundance of those bacteria in that sample. In contrast to the W5/R1525 primer pair, 70.42 % of *Myxococcales* sequences with the W2/R1525 primer pair in one Yogyakarta mangrove sample indicate high abundance of *Myxococcales*.

Thirteen major genera could be identified from three sampling sites analyzed in this study (Figure 1). The W2/R1525 primer pair could amplify sequences indicating the presence of *Cystobacter*, *Myxococcus, Stigmatella, Archangium* and *Anaeromyxobacter* of the *Cystobacterineae.* However, also *Haliangium* sequences could be amplified by this primer. The W5/R1525 primer pair amplified sequence

indicating the presence of members of the genera *Haliangium*, *Kloferia* and *Nannocystis* of the *Nannocystineae* and *Chondromyces*, *Labilithrix*, *Phaselicystis, Polyangium* and *Sandaracinus* of the *Sorangiineae.* In addition, some sequences of *Myxococcus, Stigmatella* and *Cystobacter* of the *Cystobacterineae* could be observed as being amplified by this primer.

Overall, the primers showed good specificity for their targets. Clear differences in myxobacterial diversity could be observed in the sampling sites. *Stigmatella* spp. could be observed exclusively in MK samples, Yogyakarta mangroves whereas *Nannocystis* spp., and *Labilithrix* spp. were present only in Jakarta and Yogyakarta mangroves. Only *Myxococcus* spp. were observed in all six samples, indicating that it is a common myxobacterium in mangroves samples.

3.3. Antimicrobial Activity of Myxobacteria

Twenty-five crude extracts from the 25 isolates were tested for their antimicrobial activity against ten pathogenic microorganisms, including five-Gram negative bacteria, two-Gram positive bacteria and three fungi (Figure 4). Thirteen crude extract showed activity against at least one of the human pathogenic microorganisms tested. Two of them showed activity against both Gram-negative *Escherichia coli* strains as well as against *Citrobacter freundii* and nine were active against Gram-positive *Staphylococcus aureus.* Furthermore, three of the extracts showed activity against the yeasts *Candida albicans* and *Wickerhamomyces anomalus.*

One out of thirteen active crude extracts from those myxobacteria, the crude extract of *Racemicystis* sp. strain 503MSO, which has a partial 16S rRNA sequence similarity to the type strains of ≤ 98.60 % and may constitute a novel species, was selected for further fractionation and compound identification. We considered and selected *Racemicystis* sp. strain 503MSO not only base on 16S rRNA sequence similarity to type strains but also on the missing information about compounds from genus *Racemicystis* in the myxobase database.

The crude extract of *Racemicystis* sp. strain 503MSO was fractionated in order to identify the responsible active compounds. The compound identification was done by comparing detected mass of the parent ions of the active fractions with in house myxobase database. The myxobase is a database to support research with myxobacteria, which are increasingly recognized as producers of secondary metabolites. Within myxobase, the information of bioactivity, retention time, UV spectrum, molecular mass and elemental formula of the molecule responsible for the active peak and HPLC chromatogram were provided. A summary of the active compounds from strain 503MSO was provided in Table 3, Figure S1.

Two active fractions from *Racemicystis* sp. strain 503MSO were identified comprising compounds with masses of *m/z* 375.2531 and 604.3857 $[M+H]^+$, respectively after the high-resolution mass-spectrometry (HR-MS) analysis of these active fractions. . Based on comparison of its monoisotopic mass and retention time of compound described in Myxobase, the compound with *m/z* 375.2531 $[M+H]^+$ and 604.3847 $[M+H]^+$ could not be matched to any known compound in the database yet. The structure of the mentioned active compounds can be seen in Figure S1.

Overall, this study confirmed that neglected areas such as mangroves are promising habitats for the isolation of novel myxobacteria strains and potential sources for unknown secondary metabolites producers.

4. DISCUSSION

Due to the increasing need for new antibiotics, the present study focused on the isolation of novel members of the order *Myxococcales*. Recent isolations of members of the myxobacteria have frequently resulted in the identification of new secondary metabolites with antibiotic activities. Untapped habitats such as various marine ecosystem have been proven to be a valuable resources for new microorganisms [2,38,39]. Indonesian mangroves explored in the present study were unexploited marine habitats and have a huge potential for the isolation of new uncommon species of myxobacteria.

Twenty-five strains were isolated from three different sites of Indonesian mangroves by conventional methods for isolation. *Myxococcus* sp. strains were presented in all six samples and it reflected both myxobacteria isolates versus myxobacterial communities that were amplified based on 16S rRNA gene sequences. According to the fruiting body formation of *Myxococcus* sp., they were easy to be purified and cultivated under laboratory conditiones [40]. Previously, this genus was used as model for wide-ranging studies of biotechnology [7,41–44].

The analysis of myxobacterial communities by culture-independent methods revealed more abundances than myxobacterial isolates by culture-dependent method. Moreover, some samples have predominant myxobacterial communities, for example the genus *Haliangium* was one of the most dominant and common myxobacteria in MA and MT samples. In contrast to myxobacterial communities results, the genus *Haliangium* could not be found by the culture-dependent method in our study. This could be possible because some fungal or bacterial contaminants often overgrow samples before myxobacteria can be observed. Further, the growth requirements in the laboratory might be not suitable for the rare myxobacterial species [13].

Thirteen out of twenty-five crude extracts from myxobacterial isolates yielded different activities against pathogenic microorganisms. Sharma *et al* noted that crude extracts with strong antimicrobial activities could hint to the presence of promising antimicrobial compounds against infectious disease and treatment [45]. Furthermore, these results were consistent with a previous study of Linares-Otoya *et al* [19] on antimicrobial screening of predatory bacteria from coastline. These predatory bacteria including myxobacteria from coastline may be promising sources for novel antibiotics [19].

In this study, we only focused on the active crude extract of *Racemicystis* sp. strain 503MSO, an isolate from Yogyakarta mangroves. *Racemicystis* sp. strain 503MSO was selected for further analysis because there was no information about potential bioactive compounds from the genus *Racemicystis* in the myxobase database yet. Therefore, it has a higher possibility to get new compounds from this genus.

The active fraction of *Racemicystis* sp. strain 503MSO, an isolate from Yogyakarta mangroves with *m/z* 375.2531 $[M+H]^+$ and 604.3847 $[M+H]^+$ were detected in this study and may represent so far, an undescribed compound. Therefore, *Racemicystis persica* strain 503MSO should be further explored

including substantial work for optimizing isolation, structure elucidation and biological activity of the pure substance.

Indonesian mangroves were inhabited by complex myxobacterial communities as shown by amplification using primer targeting *Cystobacterinae* (W2/R1525) and *Sorangiineae/Nannocystineae* (W5/R1525) [10,13,20]. Previously, Linares-Otoya *et al*. [19] observed that the use of universal primers (F515/R806) yielded only a neglectable amount of myxobacterial sequences (equivalent to 0.0002%) as also indicated by our work.

The rapid sequencing-based analysis of myxobacterial communities has advantages compared to previous studies comprising DGGE (Denaturing Gel Gradient Electrophoresis) [49], hybridization analysis of a 16S rRNA gene library [10] or library analysis [13,20] and by use of two PCR reactions an overview of all three suborders, i.e. *Cystobacterineae*, *Sorangiineae* and *Nannocystineae* could be obtained [50,51]. However, with the current method, a high amount of non-*Myxococcales* genera were amplified and target sequences often comprised < 10 % of sequences. Evidently, optimization of primers is still necessary. Moreover, a species level information is only possible in exceptional cases using the V5V6 hypervariable region as performed here.

Overall, mangrove habitats are a rich source for antimicrobial compounds produced by myxobacteria, highlighting the necessity to explore their diversity.

5. CONCLUSIONS

This study has highlighted the potential of isolation and characterization of myxobacteria from the Indonesian mangroves. Furthermore, it supported the use of next generation sequencing for the first time in the myxobacterial community for the three Mangroves sites. The use of cultivation techniques for the myxobacteria present in the samples and some of the crude extracts that had no antimicrobial activity against some of the tested microorganisms were limitations. In order to obtain uncultivated myxobacteria, and new bioactive compounds, we suggest further studies applying novel models of biological test and advanced techniques as well.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of this study; and in the collection, analyses, data interpretation, manuscript writing, or in the decision to publish the results.

AUTHOR CONTRIBUTIONS

Conceptualized: SO, JW, DP. Supervised: JW, DP. Performed the experiments: SO. Analyzed the data: SO, GP, LB. Wrote the paper-original draft preparation: SO. – review and edited: GP, LB, DP, TM, and JW.

REFERENCES

[1] Schäberle TF, Goralski E, Neu E, Erol Ö, Hölzl G, Dörmann P, et al. Marine myxobacteria as a source of antibiotics - Comparison of physiology, polyketide-type genes and antibiotic production of three new isolates of *Enhygromyxa salina.* Mar Drugs 2010;8:2466–79. https://doi.org/10.3390/md8092466.

[2] Schäberle TF, Lohr F, Schmitz A, König GM. Antibiotics from myxobacteria. Nat Prod Rep 2014;31:953–72. https://doi.org/10.1039/c4np00011k.

[3] Gerth K, Pradella S, Perlova O, Beyer S, Müller R. Myxobacteria: Proficient producers of novel natural products with various biological activities - Past and future biotechnological aspects with the focus on the genus *Sorangium.* J Biotechnol 2003;106:233–53. https://doi.org/10.1016/j.jbiotec.2003.07.015.

[4] Wenzel SC, Müller R. Myxobacteria - "microbial factories" for the production of bioactive secondary metabolites. Mol Biosyst 2009;5:567–74. https://doi.org/10.1039/b901287g.

[5] Landwehr W, Wolf C, Wink J. Actinobacteria and Myxobacteria---Two of the Most Important Bacterial Resources for Novel Antibiotics. In: Stadler M, Dersch P, editors. How to Overcome Antibiot. Cris. Facts, Challenges, Technol. Futur. Perspect., Cham: Springer International Publishing; 2016, p. 273–302. https://doi.org/10.1007/82_2016_503.

[6] Mulwa L, Stadler M. Antiviral Compounds from Myxobacteria. Microorganisms 2018;6:73. https://doi.org/10.3390/microorganisms6030073.

[7] Muñoz-Dorado J, Marcos-Torres FJ, García-Bravo E, Moraleda-Muñoz A, Pérez J. Myxobacteria: Moving, killing, feeding, and surviving together. Front Microbiol 2016;7. https://doi.org/10.3389/fmicb.2016.00781.

[8] Reichenbach H. The ecology of the myxobacteria. Environ Microbiol 1999;1:15–21. https://doi.org/10.1046/j.1462-2920.1999.00016.x.

[9] Dawid W. Biology and global distribution of myxobacteria in soils. FEMS Microbiol Rev 2000;24:403–27. https://doi.org/10.1016/S0168-6445(00)00032-2.

[10] Wu ZH, Jiang DM, Li P, Li YZ. Exploring the diversity of myxobacteria in a soil niche by myxobacteria-specific primers and probes. Environ Microbiol 2005;7:1602–10. https://doi.org/10.1111/j.1462-2920.2005.00852.x.

[11] Li S guang, Zhou X wen, Li P fei, Han K, Li W, Li ZF, et al. The existence and diversity of myxobacteria in lake mud - a previously unexplored myxobacteria habitat. Environ Microbiol Rep 2012;4:587–95. https://doi.org/10.1111/j.1758-2229.2012.00373.x.

[12] Brinkhoff T, Fischer D, Vollmers J, Voget S, Beardsley C, Thole S, et al. Biogeography and phylogenetic diversity of a cluster of exclusively marine myxobacteria. ISME J 2012;6:1260–72. https://doi.org/10.1038/ismej.2011.190.

[13] Mohr KI, Zindler T, Wink J, Wilharm E, Stadler M. Myxobacteria in high moor and fen: An astonishing diversity in a neglected extreme habitat.

Microbiologyopen 2017;6:1–9. https://doi.org/10.1002/mbo3.464.
[14] Jiang XT, Peng X, Deng GH, Sheng HF, Wang Y, Zhou HW, et al. Illumina Sequencing of 16S rRNA Tag Revealed Spatial Variations of Bacterial Communities in a Mangrove Wetland. Microb Ecol 2013;66:96–104. https://doi.org/10.1007/s00248-013-0238-8.
[15] Li F, Liu S, Lu Q, Zheng H, Osterman IA, Lukyanov DA, et al. Studies on antibacterial activity and diversity of cultivable actinobacteria isolated from mangrove soil in futian and maoweihai of China. Evidence-Based Complement Altern Med 2019;2019. https://doi.org/10.1155/2019/3476567.
[16] Isaka M, Suyarnsestakorn C, Tanticharoen M, Kongsaeree P, Thebtaranonth Y. Aigialomycins A-E, new resorcylic macrolides from the marine mangrove fungus Aigialus parvus. J Org Chem 2002;67:1561–6. https://doi.org/10.1021/jo010930g.
[17] Retnowati W. Identification of Streptomyces sp-MWS1 Producing Antibacterial Compounds. Indones J Trop Infect Dis 2010;1:82. https://doi.org/10.20473/ijtid.v1i2.2171.
[18] Murdiyarso D, Purbopuspito J, Kauffman JB, Warren MW, Sasmito SD, Donato DC, et al. The potential of Indonesian mangrove forests for global climate change mitigation. Nat Clim Chang 2015;5:1089–92. https://doi.org/10.1038/nclimate2734.
[19] Linares-Otoya L, Linares-Otoya V, Armas-Mantilla L, Blanco-Olano C, Crüsemann M, Ganoza-Yupanqui ML, et al. Diversity and antimicrobial potential of predatory bacteria from the peruvian coastline. Mar Drugs 2017;15:1–14. https://doi.org/10.3390/md15100308.
[20] Mohr KI, Stechling M, Wink J, Wilharm E, Stadler M. Comparison of myxobacterial diversity and evaluation of isolation success in two niches: Kiritimati Island and German compost. Microbiologyopen 2016;5:268–78. https://doi.org/10.1002/mbo3.325.
[21] Mohr K. Diversity of Myxobacteria—We Only See the Tip of the Iceberg. Microorganisms 2018;6:84. https://doi.org/10.3390/microorganisms6030084.
[22] Hoffmann T, Krug D, Bozkurt N, Duddela S, Jansen R, Garcia R, et al. Correlating chemical diversity with taxonomic distance for discovery of natural products in myxobacteria. Nat Commun 2018;9:1–10. https://doi.org/10.1038/s41467-018-03184-1.
[23] Dworkin M. Myxobacteria. Encycl. Life Sci., 2007, p. 1–5. https://doi.org/10.1002/9780470015902.a0020391.
[24] Reichenbach H. The Order *Cytophagales*. 1992. https://doi.org/10.1007/978-1-4757-2191-1_37.
[25] Hall TA. BIOEDIT: A USER-FRIENDLY BIOLOGICAL SEQUENCE ALIGNMENT EDITOR AND ANALYSIS PROGRAM FOR WINDOWS 95/98/ NT, 1999.
[26] Rath S, Heidrich B, Pieper DH, Vital M. Uncovering the trimethylamine-producing bacteria of the human gut microbiota. Microbiome 2017;5:1–14. https://doi.org/10.1186/S40168-017-0271-9.
[27] Schulz C, Schütte K, Koch N, Vilchez-Vargas R, Wos-Oxley ML, Oxley APA, et al. The active bacterial assemblages of the upper GI tract in

individuals with and without Helicobacter infection. Gut 2018;67:216–25. https://doi.org/10.1136/gutjnl-2016-312904.
[28] Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 2007;73:5261–7. https://doi.org/10.1128/AEM.00062-07.
[29] Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 2009;75:7537–41. https://doi.org/10.1128/AEM.01541-09.
[30] Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 2013;41:D590-6. https://doi.org/10.1093/nar/gks1219.
[31] Webb GI. Naïve Bayes. In: Sammut C, Webb GI, editors. Encycl. Mach. Learn., Boston, MA: Springer US; 2010, p. 713–4. https://doi.org/10.1007/978-0-387-30164-8_576.
[32] Wibowo JT, Kellermann MY, Versluis D, Putra MY, Murniasih T, Mohr KI, et al. Biotechnological Potential of Bacteria Isolated from the Sea Cucumber *Holothuria leucospilota* and *Stichopus vastus* from Lampung , Indonesia 2019.
[33] Babicki S, Arndt D, Marcu A, Liang Y, Grant JR, Maciejewski A, et al. Heatmapper: web-enabled heat mapping for all. Nucleic Acids Res 2016;44:W147-53. https://doi.org/10.1093/nar/gkw419.
[34] Okanya PW, Mohr KI, Gerth K, Jansen R, Müller R. Marinoquinolines A-F, pyrroloquinolines from *Ohtaekwangia kribbensis* (Bacteroidetes). J Nat Prod 2011;74:603–8. https://doi.org/10.1021/np100625a.
[35] Primahana G, Risdian C, Mozef T, Sudarman E, Köck M, Wink J, et al. Nonocarbolines A–E, β-carboline antibiotics produced by the rare actinobacterium *Nonomuraea* sp. From Indonesia. Antibiotics 2020;9. https://doi.org/10.3390/antibiotics9030126.
[36] Krug D, Müller R. Secondary metabolomics: The impact of mass spectrometry-based approaches on the discovery and characterization of microbial natural products. Nat Prod Rep 2014;31:768–83. https://doi.org/10.1039/c3np70127a.
[37] Reichenbach H. The ecology of the myxobacteria. Environ Microbiol 1999;1:15–21. https://doi.org/10.1046/j.1462-2920.1999.00016.x.
[38] Albataineh H, Cole Stevens D. Marine myxobacteria: A few good halophiles. Mar Drugs 2018;16. https://doi.org/10.3390/md16060209.
[39] Sun Y, Feng Z, Tomura T, Suzuki A, Miyano S, Tsuge T, et al. Heterologous Production of the Marine Myxobacterial Antibiotic Haliangicin and Its Unnatural Analogues Generated by Engineering of the Biochemical Pathway. Sci Rep 2016;6:1–11. https://doi.org/10.1038/srep22091.
[40] Zhang X, Yao Q, Cai Z, Xie X, Zhu H. Isolation and Identification of Myxobacteria from Saline-Alkaline Soils in Xinjiang, China. PLoS One 2013;8:e70466.

[41] Keane R, Berleman J. The predatory life cycle of *Myxococcus xanthus*. Microbiol (United Kingdom) 2016;162:1–11. https://doi.org/10.1099/mic.0.000208.
[42] Wallace RA, Black WP, Yang X, Yang Z. A CRISPR with roles in *Myxococcus xanthus* development and exopolysaccharide production. J Bacteriol 2014;196:4036–43. https://doi.org/10.1128/JB.02035-14.
[43] Korp J, Winand L, Sester A, Netta M. Engineering pseudochelin production in *Myxococcus xanthus*. Appl Environ Microbiol 2018;84:1–12. https://doi.org/10.1128/AEM.01789-18.
[44] Sudo S, Dworkin M. Bacteriolytic enzymes produced by *Myxococcus xanthus*. J Bacteriol 1972;110:236–45. https://doi.org/10.1128/jb.110.1.236-245.1972.
[45] Sharma P, Thakur D. Antimicrobial biosynthetic potential and diversity of culturable soil actinobacteria from forest ecosystems of Northeast India. Sci Rep 2020;10:1–18. https://doi.org/10.1038/s41598-020-60968-6.
[46] Zhang Q, Li S, Chen Y, Tian X, Zhang H, Zhang G, et al. New diketopiperazine derivatives from a deep-sea-derived *Nocardiopsis alba* SCSIO 03039. J Antibiot (Tokyo) 2013;66:31–6. https://doi.org/10.1038/ja.2012.88.
[47] Mangamuri UK, Muvva V, Poda S, Manavathi B, Bhujangarao C, Yenamandra V. Chemical characterization & bioactivity of diketopiperazine derivatives from the mangrove derived *Pseudonocardia endophytica.* Egypt J Aquat Res 2016;42:169–75. https://doi.org/10.1016/j.ejar.2016.03.001.
[48] Ding Y, Zhu X, Hao L, Zhao M, Hua Q, An F. Bioactive Indolyl Diketopiperazines from the Marine Derived Endophytic Aspergillus versicolor DY180635. Mar Drugs 2020;18. https://doi.org/10.3390/md18070338.
[49] Li B, Yao Q, Zhu H. Approach to analyze the diversity of myxobacteria in soil by semi-nested PCR-denaturing gradient gel electrophoresis (DGGE) based on taxon-specific gene. PLoS One 2014;9:1–8. https://doi.org/10.1371/journal.pone.0108877.
[50] Fanning S, Proos S, Jordan K, Srikumar S. A review on the applications of next generation sequencing technologies as applied to food-related microbiome studies. Front Microbiol 2017;8:1–16. https://doi.org/10.3389/fmicb.2017.01829.
[51] Black JS, Salto-Tellez M, Mills KI, Catherwood MA. The impact of next generation sequencing technologies on haematological research - A review. Pathogenesis 2015;2:9–16. https://doi.org/10.1016/j.pathog.2015.05.004.

TABLES

Table 1. Identities of myxobacterial isolates from Indonesian mangroves based on 16S rRNA gene sequences.
* MT: Bekasi MA: Jakarta MK: Yogyakarta

No	Next Related Type Strain	Type Strain Accession Number	Sample Name	Sample Accession Number	Similarity to Type Strain (%)	Sequence Length (bp)	Sources	Location
	Suborder *Cystobacterineae*							
1	*Archangium gephyra* DSM2261^T	DQ768106	455MSO	MW182273	98.07	883	Leaf flakes	MA
2	*Corallococcus coraloides* DSM2259^T	NR074852	82MSO	MW182281	99.66	893	Soil	MA
3	*Corallococcus coraloides* DSM2259^T	NR074852	101MSO	MW182265	99.22	896	Soil	MA
4	*Corallococcus coraloides* DSM2259^T	NR074852	412MSO	MW182280	99.16	950	Leaf flakes	MT
5	*Myxococcus fulvus* DSM16525^T	NR043946	35MSO	MW182276	98.33	897	Soil	MA
6	*Myxococcus fulvus* DSM16525^T	NR043946	191MSO	MW182272	99.55	880	Soil	MA
7	*Myxococcus fulvus* DSM16525^T	NR043946	411MSO	MW182283	99.36	932	Soil	MT
8	*Myxococcus fulvus* DSM16525^T	NR043946	511MSO	MW182268	98.20	890	Leaf flakes	MK
9	*Myxococcus fulvus* DSM16525^T	NR043946	483MSO	MW182269	99.33	891	Soil	MK
10	*Myxococcus macrosporus* DSM14697^T	NR042331	471MSO	MW182285	99.88	860	Leaf flakes	MA
11	*Myxococcus macrosporus* DSM14697^T	NR042331	161MSO	MW182282	99.78	896	Leaf flakes	MA
12	*Myxococcus macrosporus* DSM14697^T	NR042331	451MSO	MW182288	99.34	916	Leaf flakes	MA
13	*Myxococcus macrosporus* DSM14697^T	NR042331	21MSO	MW182275	98.71	928	Soil	MA
14	*Myxococcus macrosporus* DSM14697^T	NR042331	173MSO	MW182271	98.76	885	Soil	MA
15	*Myxococcus macrosporus* DSM14697^T	NR042331	421MSO	MW182284	99.70	928	Soil	MT

16	*Myxococcus macrosporus* DSM14697^T	NR042331	521MSO	MW182286	99.10	893	Sandy Beach	MK
17	*Myxococcus macrosporus* DSM14697^T	NR042331	431MSO	MW182287	99.78	929	Seaweed	MK
18	*Myxococcus macrosporus* DSM14697^T	NR042331	532MSO	MW182267	97.66	992	Soil	MK
	Suborder *Sorangiineae*							
19	*Racemicystis crocea* DSM100773^T	KT591707	503MSO	MW182266	98.30	890	Soil	MK
20	*Chondromyces robustus* DSM14608^T	AJ233942	41MSO	MW199130	95.38	763	Leaf flakes	MA
21	*Chondromyces robustus* DSM14608^T	AJ233943	151MSO	MW182279	95.34	1225	Leaf flakes	MA
22	*Chondromyces pediculatus* DSM14607^T	GU207875	61MSO	MW182274	98.52	1012	Leaf flakes	MA
	Suborder *Nannocystineae*							
23	*Nannocystis pusila* DSM53154^T	NR117463	112MSO	MW182278	99.01	905	Soil	MA
24	*Nannocystis pusila* DSM53154^T	NR117463	182MSO	MW182270	99.56	899	Soil	MA
25	*Nannocystis pusila* DSM53154^T	NR117463	16MSO	MW182277	99.45	906	Leaf flakes	MA

Table 2. The number of total reads sequences of *Myxococcales* in each sample. * MT: Bekasi, MA: Jakarta, MK: Yogyakarta

Samples	**Total Bacteria Sequences in Sample**		***Myxococcales* Sequences in Sample**		**Percentage of *Myxococcales* in Sample (%)**		**Location***
	W2/R1525	**W5/R1525**	**W2/R1525**	**W5/R1525**	**W2/R1525**	**W5/R1525**	
M39	8819	4816	437	350	4.96	7.27	MT
M41	21064	10537	315	674	1.50	6.40	MT
M44	12053	3280	204	758	1.69	23.11	MA
M45	9991	6170	56	450	0.56	7.29	MA
M48	17520	5428	12338	1467	70.42	27.03	MK
M52	13007	10743	1446	1562	11.12	14.54	MK

Total	82454	40974	14796	5261	-	-
Mean	-	-	-	-	15.04	14.27

Table 3. Compounds identified with Myxobase[1] from extracts of isolated strain 503MSO.

Crude extract of / Compound name	**In house SQL database of myxobase:**				**Experimental Data:**			
	Mono isotopic Mass	**Molecular Formula**	**RT (min)**	**Biological Source**	**Detected mass**	**Accurate mass**	**UV maxima (nm)**	**RT (min)**
Unidentified	Unknown	Unknown	Unknown	Unknown	604.3847	603.3857	278	12.94
Unidentified	Unknown	Unknown	Unknown	Unknown	375.2531	374.2543	240	16.05

*RT is retention time. [1]Myxobase in-house database of myxobacteria group

FIGURE

Figure 1. Three different sampling sites from Indonesian mangroves including MA: Mangrove Muara Angke, North Jakarta, MT: Mangrove Muara Tawar, Bekasi, MK: Mangrove Api-Api, Yogyakarta. This map was adapted from www.d-maps.com

Figure 2. Some myxobacteria isolated from Indonesia Mangrove with swarming area of strain 503MSO on VY/2 agar medium, fruiting bodies of strains 455MSO, 412MSO, 413MSO, 451MSO on VY/2 agar medium, and strain 151MSO on Stan21 agar medium.

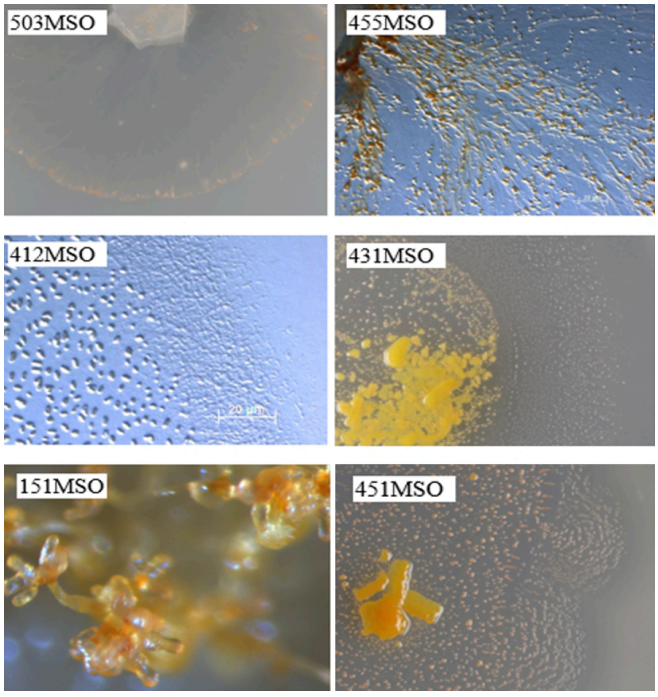


Figure 3. Relative abundances of myxobacterial genera in Indonesian Mangroves. MT: Bekasi MA: Jakarta MK: Yogyakarta using different set of primer W2/R1525 and W5/R1525. The community composition was revealed using primers targeting the suborder *Cystobacterineae* and the suborders *Sorangiineae/Nannocystineae*, respectively.

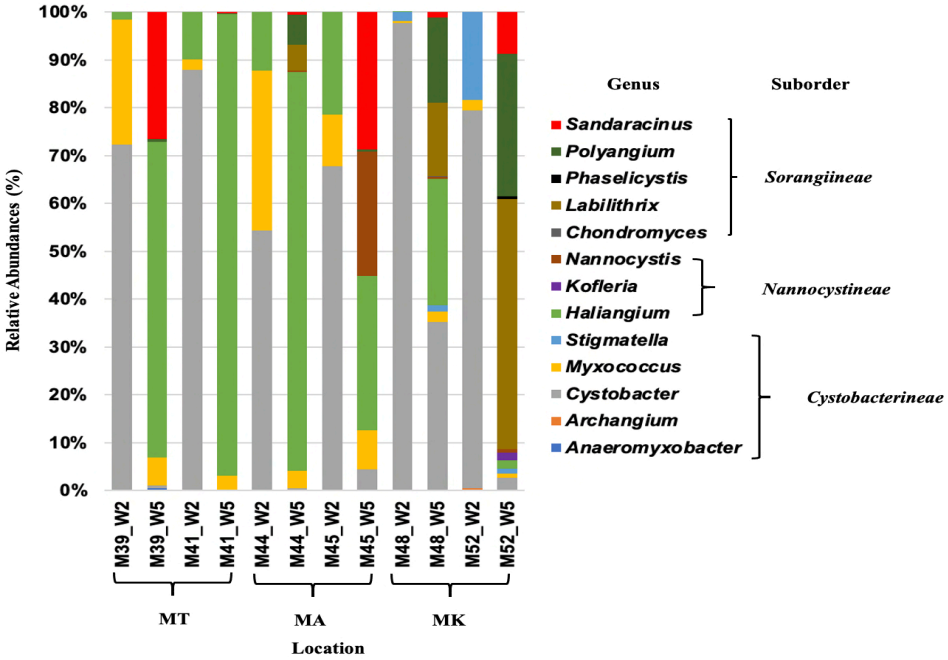
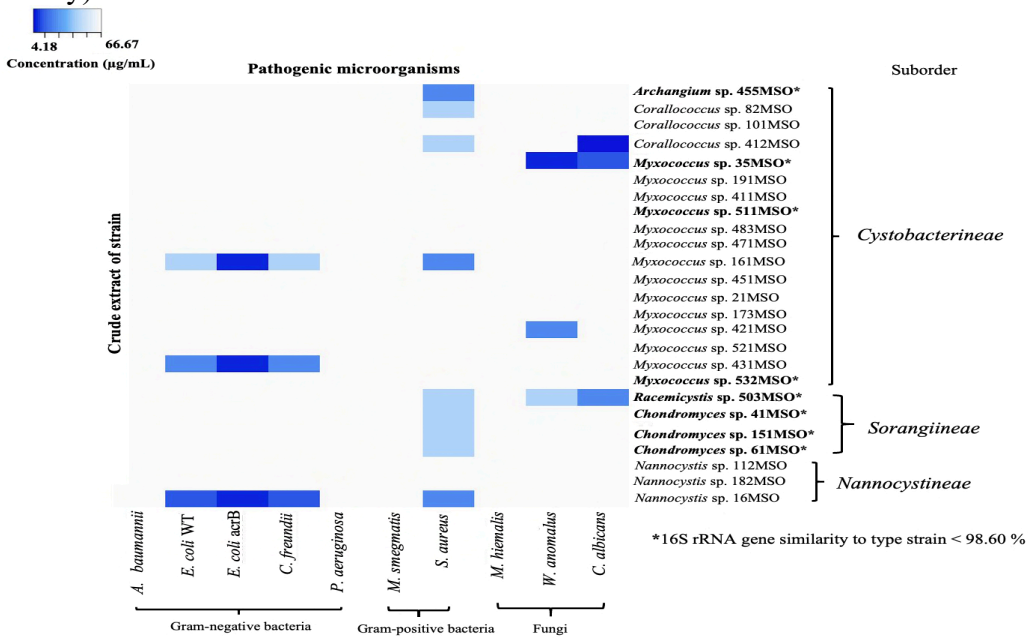


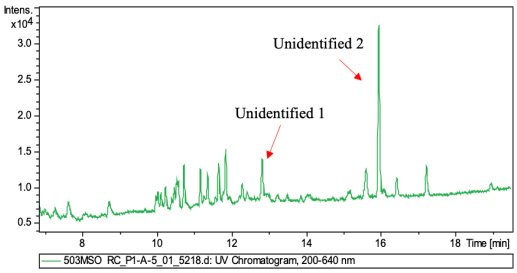
Figure 4. The antimicrobial activity of crude extracts from 25 myxobacterial isolates (Y-axis) acting against 10 different microbial pathogens (X-axis). The different colors indicate different minimum inhibitory concentrations (MIC) of crude extracts exerting inhibitory effects. MIC values were calculated by serial dilution with the highest concentration of 66.7 µg/mL (weak antimicrobial activity) diluted 8 times to the lowest concentration of 0.52 µg/mL (strong antimicrobial activity).

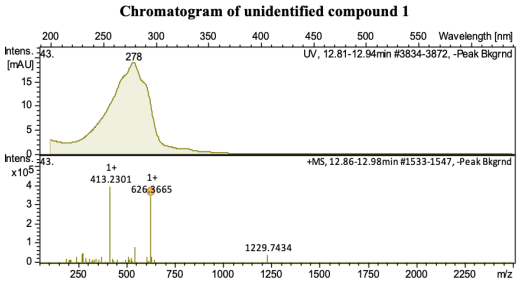


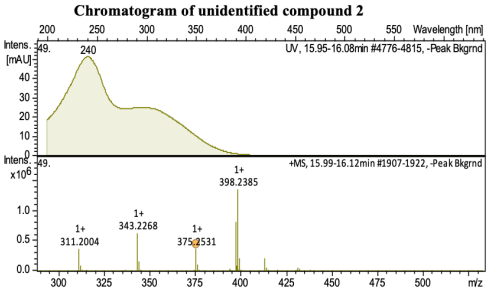
SUPPORTING INFORMATION

The following data are available online

Figure S1. Structure of compound and their identification in crude extract of *Racemicystis* sp. strain 503MSO.







Supplementary 7

Taxonomy and Antimicrobial Activity of Gliding Bacterium from Indonesian Mangroves

SENLIE OCTAVIANA[1,2], TJANDRAWATI MOZEF[3], JOACHIM WINK[1]

[1]Microbial Strain Collection, Helmholtz Center for Infection Research, 38124, Braunschweig,Germany. Email: Senlie.Octaviana@helmholtz-hzi.de, Joachim.Wink@helmholtz-hzi.de
[2]Research Center for Biotechnology, Indonesian Institute of Sciences, Cibinong, 16911, Indonesia. Email: senl001@lipi.go.id
[3]Research Center for Chemistry, Indonesian Institute of Sciences, Serpong, 15314, Indonesia. Email: tjandrawati@lipi.go.id

Abstract
The discovery of new antibiotic is needed, due to the increasing antimicrobial resistance in nature. Therefore, this study aims to isolate gliding bacteria and to ascertain their antimicrobial activity against pathogenic microbes. This research was conducted by isolating gliding bacteria from mangrove sediment in Muara Angke, North Jakarta, Indonesia. All of the strains were identified by 16S rRNA genes sequencing and those selected strain was characterized using polyphasic approach. The performance of crude extracts against to ten pathogenic microorganisms were detected using serial dilution test in 96-well plates. Mangrove gliding bacteria isolates designated 313MSO and 314MSO were showed high homology to *Ohtaekwangia kribbensis* with 96.20% and 99.12% similarity, respectively. The polyphasic approach led to the conclusion that the strain 313MSO was a new species of the genus *Ohtaekwangia*. Twenty-three crude extracts were obtained from cultivating the strain in twenty-three different media. The most of them inhibited the growth of *Staphylococcus aureus* Newman with the minimum concentration of 33.33-66.67 µg/ml. Four compounds (Marinoquinolines A-D) were obtained from HPLC-MS analysis, which were previously isolated by Okanya *et al* (2011). Furthermore, the strain 313MSO is presently being studied for in-depth identification of additional unknown metabolites detected in the crude extracts.
Keywords: Antimicrobial, Indonesia, mangrove, gliding bacteria.

1. Introduction

Since Reichenbach in 1981 introduced the taxonomy of gliding bacteria, these bacteria have more attention for harboring different natural products. Gliding bacteria are Gram-negative bacteria, which can glide on surfaces and are grouped into the *Flexibacteriae* class, which includes two classification orders namely, *Cytophagales* and *Myxobacterales*. Nowadays, it is distinguished in the phylum level, as those without fruiting bodies belong to the phylum *Bacteriodetes*, while those possessing this feature belong to *Myxococcales*.

Over the past decades, members of phylum *Myxococcales* have proven to be promising source of new bioactive metabolites. Therefore, around six hundred

derivatives and a hundred new carbon skeleton metabolites have been isolated and identified. These bioactive metabolites exhibit antibacterial, antifungal, antimalarial, antioxidative and antiviral activities (Gerth *et al.*, 2003; Wenzel and Müller, 2009; Landwehr *et al.,* 2017; Mulwa and Stadler, 2018). Meanwhile, inspite of the general outstanding in the production of diverse bioactive metabolite, the discovery of new antibiotic producers in nature is still needed.

Gliding bacteria are commonly found in various habitats (Dawid, 2000). In 2011, *Ohtaekwangia* which a new genus of gliding bacterium, was found in Korea marine sand, belonging to the *Cythophagales* order, which was previously proposed as a novel species of *Ohtaekwangia koreensis* (3B-2^T) and *Ohtaekwangia kribbensis* (10AO^T) (Yoon *et al*., 2011). In the same year, Okanya *et al.* (2011) isolated Marinoquinolines A and five derivatives B-F from a strain, which is closely related to *Ohtaekwangia kribbensis*. These compounds reacted against pathogenic microorganism and also show cytotoxicity to growing mammalian cell lines.

Sangnoi *et al* (2016) gave a report on novel genera of gliding bacteria, *Flavobacterium* and *Lysobacter* from a conservation site at Cheow Land Reservoir in southern Thailand. Moreover, all of these strains have antibacterial activities when tested with agar-well diffusion assay. The purpose of this study is to isolate and to identify gliding bacteria from Indonesian samples using 16S rRNA gene sequences. Initially, these bacteria were found as a contaminant in the agar plates for isolation of myxobacteria. Nevertheless, one of the new strains was selected for in-depth analyses and characterization using the polyphasic approach, and evaluating their capability in the production of secondary metabolite, especially for antimicrobial activity.

2. Material and Methods

2.1.Sampling area

Samples from Mangrove sediments were taken onto sterile zip-log plastic bag from Taman Konservasi Muara Angke (6º105321'N 106º735578'E), Jakarta, Indonesia. All the samples were dried at 30ºC and stored at room temperature.

2.2.Isolation of Gliding Bacterium

The strains were isolated using the methods of Shimkets *et al* (2006) and placed on water-agar medium where their surfaces were carefully cross-streaked with living *Escherichia coli* K12. After five days, swarm colonies were observed using a dissecting microscope and transferred with a sterile needle into fresh water-agar with dead *Escherichia coli.* The isolates were typical gliding bacteria without fruiting bodies growing faster than myxobacteria. Pure cultures were then transferred into 20 ml CY/H liquid medium [per liter: 1.0 g defatted soy flour, 1.0 g glucose, 4.0 g starch (Cerestar), 1.5 g yeast extract, 1.5 g casitone, 1.0 g $CaCl_2 \cdot 2H_2O$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.008 g iron EDTA, 11.8 g HEPES, pH 7.3]. While 100 ml CY/H liquid medium of well-grown culture and 1.5 ml portion of them were conserved at -80ºC.

2.3.Identification of Pure Cultures by 16S rRNA Sequences

The DNA from pure cultures were extracted using Invitek Spin Plant Mini Kit (Invisorb) following the manufacturer's instruction. The PCR reaction of 16S rRNA was performed in a Mastercycler Gradient (Eppendorf, Hamburg, Germany) following Mohr *et al.* (2015, 2017). The PCR products were then examined using electrophoresis on agarose (0.8 %) and purified with NucleoSpin Gel and PCR Clean up Kit (Macherey-Nagel, Düren, Germany).

The pure PCR products were sent to the sequence service at HZI (Helmholtz Centre for Infection Research). The 16S rRNA gene sequences were assembled with the BioEdit programme (Hall, 1999) and compared with the public database NCBI using BLAST search (Altschul *et al*., 1990). The phylogenies were inferred by the GGDC web server (Meier-Kolthoff *et al.,* 2013a) at http://ggdc.dsmz.de/ using the DSMZ phylogenomics pipeline of a single gene (Meier-Kolthoff *et al.,* 2014). All of the sequences were aligned using MEGAX software. Maximum likelihood (ML) and maximum parsimony (MP) trees were inferred from the alignment with RAxML (Stamatakis 2014) and TNT (Goloboff *et al*, 2008), respectively. Based on the ML, rapid bootstrapping with the autoMRE criterion (Pattengale *et al*, 2010) and subsequent search for the best tree were used. While for MP, a thousand bootstrapping replicates were used in conjunction with tree-bisection-and-reconnection branch swapping and ten random sequence addition replicates. Lastly, the sequences were checked for a compositional bias using the X^2 test as implemented in PAUP* (Swofford 2002).

2.4.Morphological Identification

The morphology and cell size from agar plates were observed using microscope (AxioCam MRc9) at a magnification 1000x. Approximately ten to twenty randomly selected cells were measured (length) by using Axio-Vision Rel. 4.8 software followed by defining the Gram staining based on Sigma Aldrich manufacturer's instructions.

2.5.Physiological Identification

Different media namely R2A, VY/2, CY and VY/4 ASW were selected and inoculated with 10 µl well-grown cultures. After been incubated at 30°C for five days, the plated were checked by visual inspection and the growth was characterized by measuring the swarming colonies. The optimal temperatures for their growth were determined after being incubated at 10°C, 20°C, 25°C, 28°C, 30°C, 34°C, 40°C and 44°C. Moreover, the pH following 4; 5; 6.5; 7.5; 8.5 and 9 were observed for optimal growth. In the analysis of sodium tolerance, 10 µl well-grown cultures were inoculated on optimal conditions as mentioned above with different concentration of NaCl (0; 2; 5; 7.5 and 10%). Therefore, their entire physiological characteristic was observed by visual inspection.

2.6.Chemotaxonomy Identification

The flexirubin pigment played an important role within the members of *Cytophagales* and analyzed following Reichenbach (1981) instructions. Fifty mg of dry cell mass were prepared and fatty acid methyl ester extraction following the Microbial Identification System (MIDI) protocol. The fatty acids were then analysed using the GC-Chromatography, while its phospholipid was

defined according to Carthwight (1993) instructions. The catalase and oxidase tests were observed following Sigma Aldrich protocol, when both of experiment tested positive, they indicated bubble formation and discoloration to purple-blue, respectively. The characteristic of the enzymes was defined using APIZYM and APICAMPY, while the Gen III BIOLOG System was used for the assimilation substrates. Then, antibiotic resistances were checked using various antibiotics (50 mg/ml) and observed by visual inspection after five days.

2.7.Screening for Antimicrobial Activity

The selected pathogenic microorganism used in this antimicrobial study were *Escherichia coli* WT-BW 25113, *Escherichia coli* JW0451-02, *Acinetobacteri baumanii* DSM30008, *Pseudomonas aeruginosa* Pa14, *Staphyl ococcus aureus* Newmann, *Citrobacter freundii* DSM 30039, *Mycobacterium smegmatis* ATCC 700084, *Mucor himalis* DSM 2656, *Pichia anomala* DSM 6766 and *Candida albicans* DSM 1665. All were obtained from microbial strain collection group, HZI, Germany. The details for the culture conditions were mentioned in Supplementary 1.

The well-grown cultures of the gliding bacterium strain were prepared with 100 ml CY/H liquid medium and 10 % of them was then inoculated in twenty-three different media (Supplementary 2) with XAD-adsorber resin. After five days, the cultures were filtered and were extracted with 70 ml acetone. After the solvent was evaporated, 1 ml MeOH was added and stored at -20ºC.

The crude extracts were evaluated for antimicrobial activity in the serial dilution test using 96-well plates with ten different pathogenic microbes. After overnight incubation, the antimicrobial activity was evaluated by visual inspection. A clear zone indicated the crude extract inhibition of the pathogenic microorganism, while an overgrown of microbe indicated a negative inhibition activity.

The de-replication process was followed by using a fractionation to identify the active compounds. Therefore, the active crude extracts were analyzed by preparative HPLC, HPLC/MS and Brucker Data Analysis and were compared with the in-house database “myxobase” and/or DNP (dictionary of natural product). Then, the active compounds were identified as “new” or “known” compounds.

2.8.Nucleotide sequences accession number

The GenBank/EMBL/DDBJ accession numbers for the partial 16S rRNA gene sequences of 313MSO and 314MSO strains were defined as MT591272 and MT591273, respectively.

3. Results

3.1.Strain Isolation

The two strains of gliding bacteria from Indonesian habitat were isolated (Table 1), purified, and grew fast after three days of inoculation. The 313MSO and 314MSO strains were isolated from Mangrove sediment, and compared to the partial 16S rRNA gene sequences using BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi). These strains were identified as *Ohtaekwangia* spp. with the similarity of 96.20 and 99.12%,

respectively, while 313MSO strain had lower similarity (96.20%) than intraspecies. Therefore, it was selected for in-depth study.

A phylogenetic analysis of 16S rRNA gene sequences from strain 313MSO was shown in Figure. 1. The input nucleotide matrix comprised 45 operational taxonomic units and 1449 characters, 713 of which were variable and 545 of which were parsimony-informative. The base-frequency check indicated no compositional bias ($p = 0.68$, $\alpha = 0.05$). The Maximum Likelihood (ML) analysis under the GTR+GAMMA model yielded a highest log likelihood of -16906.77, while the estimated alpha parameter was 0.27. The ML bootstrapping did not converge, hence a thousand replicates were conducted; and the average support was 63.43%. The Maximum Parsimony (MP) analysis yielded the best score of 3473 (consistency index 0.37, retention index 0.57), twenty-two best trees and MP bootstrapping average support was 70.86%.

The levels of partial 16S rRNA gene sequence similarity between strain 313MSO and other bacterial taxa are acceptable to differentiate this strain from known genera of the phylum *Bacteroidetes*. It should be classified in a new species within the genus *Ohtaekwangia*.

3.2.Characteristic of The New Strain

The gliding bacterial strain of 313MSO was characterized and compared with the strains of *Ohtaekwangia kribbensis* 3B-2^T and *Ohtaekwangia koreensis* 10AO^T. A typical colony bright yellow in colour with radial patterns were observed (Figure 2). As described in Table 3, the morphological characteristics of strain 313MSO were Gram-negative and rod-shaped with the optimum size of 1-5 mm. This strain showed the optimum growth in VY/2 and R2A medium. It preferably grew in pH ranging 6.5 to 7.5 at 30°C on agar plates. The yeast extract is better than carbohydrate for stimulating the growth of most of the members of *Cytophagales* order.

The 313MSO strain grew well in 0-1% NaCl and has no catalase and oxidase enzyme activities, and also flexirubin type pigments. However, the reference strains have these features. For antibiotic resistances, the strain showed resistance to gentamycin, however, susceptible to polymycin, kanamycin, ampicillin and chloramphenicol (not included in Table 1). Although the strain had different morphological properties with references strains, it has no strength defined to the new species.

The biochemical properties of strain 313MSO were shown in Table 2. The following products were assimilated by this strain namely, acetoacetic acid, alpha-hyydroxy-butyric acid, D-lactic acid methyl ester, D-serine, glycerol, L-alanine, L-lactic acid, methyl pyruvate, myo-inositol, N-acetyl neuraminic acid, and sodium butyrate, however, were not produced by the reference strains. As mentioned in Table 3, the 313MSO strain showed no enzyme activities for alpha fucosidase, esterase and HIP-purate, however, indicated close relationship to *Ohtaekwangia kribbiensis* 3B-2^T, based on APIZYM and APICAMPY tests.

The 313MSO strain also showed different characters of fatty acid properties compared to references strains. As mention in Table 3, the analyses of fatty acids were performed using MIDI protocol. The 313MSO strain had C18:0 straight-chain fatty acid and C16:0 iso brached fatty acid. Lastly,

the strain 313MSO had different characters of the biochemical properties compared to the reference strains.

3.3.Antimicrobial Activity

The twenty-three crude extracts were examined for antimicrobial activity towards pathogenic microbes which included the five Gram-negative, two Gram-positive bacteria and three fungus activities (Figure 4). The antimicrobial assay (MIC) showed that most of the crude extracts exhibited growth inhibition against *Staphylococcus aureus* Newmann, including the strain 313MSO when cultured in P medium and 1/10 CY/H medium with the minimum concentration of 33.33 µg/ml. The 313MSO strain also showed the inhibition activity against *Escherichia coli* acrB JW0451-2, when cultured in 1/10 E medium and against *Pseudomonas aeruginosa* Pa14 with the minimum concentration of MIC 66.67 µg/ml, when cultivated in MA medium. However, all of the crude extracts had no inhibition towards *Candida albicans* DSM1665, *Acinetobacter baumannii* DSM30008, *Esch erichia coli* WT BW25113, *Citrobacter freundii* DSM30039, *Mycobacterium smegmatis* ATCC70 0084, *Mucor himalis* DSM2656 and *Pichia anomala* DSM6766.

4. Discussion

Two gliding bacteria strains of 313MSO and 314MSO were isolated from mangrove sediment in North Jakarta, Indonesia. The differences in the gliding bacteria and myxobacterial strain were difficult to define, due to the unstable nature of the fruiting bodies in myxobacteria, which were lost during purification (Zhang *et al*., 2013). Furthermore, the comparison of 16S rRNA gene sequences was useful for identifying closely related strains. The 313MSO strain from mangrove showed the lowest similarity (96.20%) to this species.

All of the gliding bacteria were isolated during a routine myxobacterial isolation project in the laboratory. The 313MSO strain was selected for in-depth study. It could optimally grow be grow in VY/2 medium, while reference strains grow in R2A medium (Table 1). A polyphasic study with the 16S rRNA gene sequence analysis of this strain showed different characters (Tables 1; 2; 3), also, the phylogenetic tree clearly exhibited separation from the reference strains (Figure 1). Therefore, it was proposed that novel should have similarity percentage below 98.65%. The determination of full genome sequences included G+C content, DNA-DNA hybridization and quinone production should be the subject of a further study.

This is the first report describing a novel taxonomy using the polyphasic approach and their potential for antimicrobial activity from unexplored Indonesian habitat, especially in the mangrove forest. These results provide new information about the ecology of a species of the gliding bacterium belonging to genus *Ohtaekwangia*. Moreover, the methodologies for the antimicrobial screening are useful for further studies of the economically important organisms.

The twenty-three crude extracts that were obtained from the 313MSO strain that were cultivated in different media (Figure 2) and most of them had specific

inhibition towards *Staphylococcus aureus* Newmann with the minimum concentration 33.33-66.67 µg/ml. The 313MSO strain had stronger antibacterial activity towards pathogenic bacteria compared to the aquatic gliding bacteria with the minimum concentration 75 µg/ml (Sangnoi *et al*., 2016).

Base on de-replication with preparative HPLC and HPLC/MS, the active crude extracts from Pol and 1/10 CY/H medium with the minimum concentration MIC 33.33 µg/ml belonged to the fatty acid group. Moreover, Marinoquinolines A and five derivates (B-F) were also detected from both media. Even though Okanya *et al.* (2011) isolated Marinoquinolines A and five derivates (B-F) using PWu25, which belong to *Ohtaekwangia* sp. in E broth medium, in the present study 313MSO strain, showed no antimicrobial activity in the same medium. Consequently, it is suggested that the strain 313MSO could be cultivated on other different media (optimization media for examples) and/or co-cultivated with microbial natural product producers for stimulating secondary metabolite. Moreover, all of the crude extracts may be analysed in other biological test models.

In conclusion, the mangrove is an ecosystem that harbours gliding bacteria, since it is an unusual environment between the marine and terrestrial habitat. The 313MSO strain was classified as a new species, when it showed different character from references strain within genera *Ohtaekwangia.* Their crude extracts showed specific antimicrobial activity towards Gram-positive pathogenic bacteria.

Conflicts of interest

The authors declare that there is no conflict of interest on this research.

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References

Altschul SF *et al*. 1990. Basic local alignment search tool. *Journal Of Molecular Biology* 215: 403-410.

Cartwright. 1993. Separation and analysis of phospholipids by thin layer chromatography. *Methods Mol Biol*. 19:153-67.

Dawid W. 2000. Biology and global distribution of myxobacteria in soils. *FEMS Microbiol Rev*. 24: 403–427.

Gerth K *et al*. 2003. Myxobacteria: proficient producers of novel natural products with various biological activities--past and future biotechnological aspects with the focus on the genus Sorangium. *J Biotechnol*. 106 (2-3):233-53.

Goloboff PA *et al*. 2008. TNT, a free program for phylogenetic analysis. *Cladistics*. 24: 774-786. doi:10.1111/j.1096-0031.2008.00217.x.

Hall TA. 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95-98.

Landwehr W *et al*. 2016. Actinobacteria and Myxobacteria-Two of the Most Important Bacterial Resources for Novel Antibiotics. *Microbiology and Immunology*. Doi: 10.1007/82_2016_503

Meier-Kolthoff JP *et al*. 2013a. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics*. 14: 60. Doi: 10.1186/1471-2105-14-60.

Meier-Kolthoff JP *et al*. 2014. Complete genome sequence of DSM 30083T, the type strain (U5/41T) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. *Stand Genomic Sci*. 10: 2. Doi: 10.1186/1944-3277-9-2.

Mohr KI *et al*. 2015. Comparison of myxobacterial diversity and evaluation of isolation success in two niches: Kiritimati Island and German Compost. *Microbiology Open*. 1-11.

Mohr KI *et al*. 2017. Myxobacteria in high moor and fen: an astonishing diversity in a neglected extreme habitat. *Microbiology Open*. 6(4): e00464.

Mulwa L, Stadler M. 2018. Antiviral compounds from Myxobacteria. *Microorganism*. 6(3): 73.

Okanya PW *et al*. 2011. Marinoquinolines A−F, Pyrroloquinolines from *Ohtaekwangia kribbensis* (Bacteroidetes). *J. Nat. Prod*. 74 (4): 603-608.

Pattengale ND *et al*. 2010. How many bootstrap replicates are necessary. *J Comput Biol*. 17: 337–354.

Reichenbach H. 1981. Taxonomy of the gliding bacteria. *Ann. Rev. Microbiol*. 35:339-634.

Sangnoi Y *et al*. 2016. Antibacterial activity of aquatic gliding bacteria. *Springerplus*. 5:116.

Shimkets LJ *et al*. 2006. The myxobacteria. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K. H., and Stackebrandt, E. (Eds). The Prokaryotes: A Handbook on the Biology of Bacteria, 3rd edn, vol. 7, pp. 31–115. New York: Springer.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 30: 1312-1313. doi:10.1093/bioinformatics/btu03.

Swofford DL. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0 b10. Sinauer Associates.

Wenzel SC, Müller R. 2009. Myxobacteria-'microbial factories' for the production of bioactive secondary metabolites. *Mol Biosyst*. 5(6):567-74. doi: 10.1039/b901287g.

Yoon J-H *et al*. 2011. *Ohtaekwangia koreensis* gen. nov., sp. nov. and *Ohtaekwangia kribbensis* sp. nov., isolated from marine sand, deep-branching members of the phylum Bacteroidetes. J of Systematic and Evolutionary Microbiology. 2011. 61:1066-1072.

Zhang X *et al*. 2013. Isolation and identification of myxobacteria from Saline-Alkaline Soils in Xinjiang, China. *Plos One,* 8(8), E70466. Doi: 10.1371/Journal.Pone.0070466.

Figures

Figure 1. Maximum Likelihood (ML) tree inferred under the GTR+GAMMA model and rooted by outgroup-rooting. The branches are scaled in terms of the expected number of substitutions per site. The numbers above the branches are supporting values when larger than 60% from ML (left) and MP (right) bootstrapping.

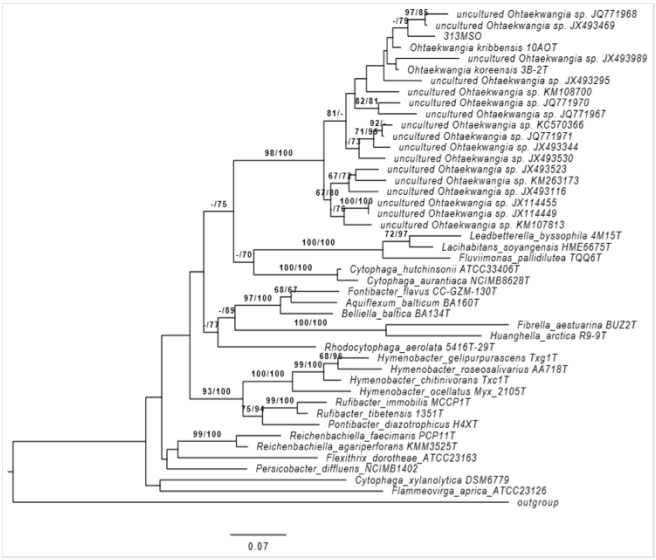

Figure 2. Morphological characteristic of gliding bacterium. Strain 313MSO was growth on VY/2 agar (A) and observed under optical microscope (B). The scale bars are 10-20 µm.

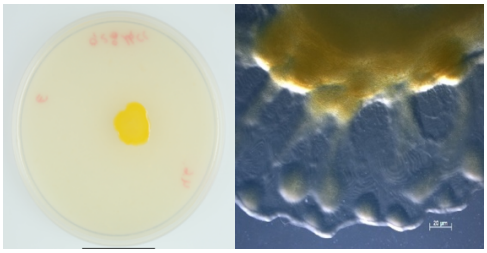

Figure 3. Diagram of antimicrobial activity from the strain 313MSO isolated in twenty-three different media that were tested against to ten different pathogenic microbes. The letters is designated by letters (A = *Candida albicans* DSM1665, B = *Acinetobacter baumannii* DSM30008, C = *Escherichia coli* WT BW25113, D = *Escherichia coli* acrB JW0451-2, E = *Citrobacter freundii* DSM30039, F = *Mycobacterium smegmatis* ATCC700084, G = *Pseudomonas aeruginosa* Pa14, H = *Staphylococcus aureus* Newmann, I = *Mucor himalis* DSM2656, J = *Pichia anomala* DSM6766). The different colour indicates the antimicrobial activity: the darker the red colour, the stronger inhibition activity, which correlates to the lower crude extract concentration.

Tables

Table 1. Morphological Properties of Strain 313MSO

Characteristic	313MSO	3B-2T*	10A0-T*
Cell Shape	Rod	Rod	Rod
Cell Size [µm]	1.0-5.0	1.0-5.0	1.5-7.5

NaCl Tolerances [w/v]			
0-1%	+	+	+
2,5-10%	-	-	-
Catalase	-	+	+
Oxidase	-	+	+
Flexirubin type pigment	-	+	+
Optimal growth	30°C	30°C	30°C
Optimal pH	6.5-7.5	6.5-7.5	6.5-7.5
Optimal medium	VY/2	R2A	R2A
Gram Staining	negative	negative	negative
Antibiotic Resistance			
Gentamycin [50µg/ml]	+	-	+
Phospholipid	Phosphatidy linositol-mannosid	Phosphatod ylethanola mine	Phosphato dylethanol amine

*Note: + able; – unable; 3B-2T = O. *kribbiensis*; 10A0-T = O. *koreensis*

Table 2. Biochemical Properties of New Strain

Characteristic	313MSO	3B-2T*	10A0-T*
Assimilation of (Biolog)			
3-Methyl Glucose	-	+	-
Acetic Acid	+	-	+
Acetoacetic Acid	+	-	-
Alpha-Hyydroxy-Butyric Acid	+	-	-
Alpha-Keto-Glutaric Acid	-	-	+
Beta-Hydroxy-D, L-Butyric Acid	-	-	+
Beta-Methyl-D-Glucosidase	-	+	+
Bromo-Succinic Acid	-	-	+
Citric Acid	-	-	+
D-Aspartic Acid	-	+	+
D-Fructose-6-PO4	+	-	+
D-Galactose	+	-	+
D-Gluconic Acid	+	-	+
D-Lactic Acid Methyl Ester	+	-	-
D-malic Acid	-	-	+
D-Mannose	+	-	+
D-Saccharic Acid	-	-	+
D-Serine	+	-	-
Formic Acid	-	-	+
Gentiobiose	+	-	+
Glucuornicamide	+	+	+
Glucuronic Acid	-	-	+
Glycerol	+	-	-
Glycyl-L-Proline	-	+	+

Guanidine HCl	-	-	+
L-Alanine	+	-	-
L-Arginine	-	-	+
L-Fucose	+	+	+
L-Galactonic Acid Lactone D-Gluconic Acid	-	+	+
L-Glutamic Acid	+	-	+
Linomycin	+	-	+
Lithium Chloride	+	-	+
L-Lactic Acid	+	-	-
L-Malic Acid	-	-	+
L-Rhamnose	+	-	+
L-Serine	+	-	+
Methyl Pyruvate	+	-	-
Minocycline	+	-	+
Mucic Acid	-	-	+
myo-Inositol	+	-	-
N-Acetyl Neuraminic Acid	+	-	-
N-Acetyl-D-Galactosamine	+	-	+
Nalidixic Acid	+	-	+
p-Hydroxy-Phenylacetic Acid	-	-	+
Potassium Tellurite	+	-	+
Propionic Acid	-	-	+
Quinic Acid	-	+	+
Sodium Butyrate	+	-	-
Sucrose	+	-	+
Tetrazolium Violet	-	-	+
Tetrazolum Blue	+	-	+
Troleandomycin	+	-	+
Vancomycin	+	-	+
APIZYM			
Trypsin	w	+	-
Naphtol-AS-B1-Phosphohydrolase	+	+	-
Beta glucosidase	+	+	-
Alpha fucosidase	-	+	-
API CAMPY			
Esterase	-	+	-
HIP-purate	-	+	-
Gamma Glutamyl Transferase	+	-	-

*Note: + able; – unable; w = weak; 3B-2T = O. *kribbiensis*; 10A0-T = O. *koreensis*

Table 3. Major Fatty Acid of the New Strains

Fatty Acid	313MSO	3B-2T*	10A0-T*
Straight-chain			

$c_{15:0}$	-	-	2,1
$c_{16:0}$	-	-	22,2
$c_{18:0}$	1,59	-	-
Branched			
Iso $c_{14:0}$	-	-	1,7
Iso $c_{15:0}$	-	20,4	30,2
Iso $c_{16:0}$	3,8	9,5	4
Iso $c_{17:0}$	-	-	7,4
Unsaturated			
$c_{16:1}$ ω7c	-	55,2	27,4
Unknown			
ECL 11.864	-	6,2	3,3
ECL 22.207	-	8,7	1,7

*Note: 3B-2T = O. *kribbiensis*; 10A0-T = O. *koreensis*

APPENDIX

Table A1. MOBio PowerSoil DNA Kit

Solution	Composition
C1	SDS
C2	Reagent to precipitate non-DNA organic and inorganic material
C3	Reagent to precipitate non-DNA organic and inorganic material
C4	High salt concentration
C5	Ethanol
C6	Elution Buffer

Table A2. Barcodes of primer pair 807R/1050F

	Forward
	AATGATACGGCGACCACCGAGATCTACACTAAGTTCCTCTT
I_A506	TCCCTACACGACGCTCTTCCGATCT
	Reverse
	CAAGCAGAAGACGGCATACGAGATATCACGACGTGACTGG
I_A701	AGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGATACAGTGGTGTGACTGG
I_A702	AGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGATCAGATCCAGTGACTGG
I_A703	AGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGATACAAACGGGTGACTGG
I_A704	AGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGATAACCCCTCGTGACTGG
I_A706	AGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGATCCCAACCTGTGACTGG
I_A707	AGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGATCACCACACGTGACTGG
I_A708	AGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGATGAAACCCAGTGACTGG
I_A709	AGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGATTGTGACCAGTGACTGG
I_A710	AGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGATAGGGTCAAGTGACTGG
I_A711	AGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGATAGGAGTGGGTGACTGG
I_A712	AGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGATATTACTCGGTGACTGG
I_D701	AGTTCAGACGTGTGCTCTTCCGATCT

Table A3. Screening medium within in this Study

Screening Medium		Composition	Notes
A-Medium	0.40 %	glycerol (87 % w/v)	pH 7,4
	0.40 %	soy flour (degreased)	
	0.20 %	yeast extract (Marcor type 9000)	
	0.10 %	MgSO4 · 7H2O	
	50 mM	HEPES (11,9g/l)	
	8 mg/lL	Fe-EDTA	
	0.80 %	starch (Cerestar)	
	0.10 %	$CaCl_2$.$2H_2O$	
CLF-Medium	0.40 %	fructose monohydrate	pH 7,0
	0.60 %	glucose monohydrate	
	1 %	skim milk	
	0.20 %	yeast extract (Marcor type 9000)	
	0.10 %	$CaCl_2$.$2H_2O$	
	0.10 %	MgSO4 · 7H2O	
	50 mM	HEPES (11,9g/l)	
CY-Medium	0.30 %	Castione	pH 7,2
	0.10 %	yeast extract (Marcor type 9000)	
	0.10 %	$CaCl_2$.$2H_2O$	
	50 mM	HEPES (11,9g/l)	
	1.60 %	Agar (Difco)	
E - Medium	0.40 %	skim milk	pH 7,4
	0.40 %	soy flour (degreased)	
	0.20 %	yeast extract (Marcor type 9000)	
	1 %	starch (Cerestar)	
	0.10 %	MgSO4 · 7H2O	
	50 mM	HEPES (11,9g/l)	
	8 mg/L	Fe-EDTA	
	0.50 %	glycerol (87% w/v)	
H-Medium	0.20 %	soy flour (degreased)	pH 7,4
	0.20 %	glucose monohydrate	
	0.80 %	starch (Cerestar)	
	0.20 %	yeast extract (Marcor type 9000)	
	0.10 %	$CaCl_2$.$2H_2O$	
	0.10 %	MgSO4 · 7H2O	
	50 mM	HEPES (11,9g/l)	

	8 mg/L	Fe-EDTA	
M-Medium	1 %	peptone (soy)	pH 7,2
	1 %	maltose monohydrate	
	0.10 %	CaCl2 .2H2O	
	0.10 %	MgSO4 · 7H2O	
	50 mM	HEPES (11,9g/l)	
	8 mg/L	Fe-EDTA	
POL - Medium	0.30 %	Probion	pH 7,2
	0.30 %	Starch (Cerestar)	
	0.05 %	$CaCl_2$.$2H_2O$	
	0.20 %	MgSO4 · 7H2O	
	50mM	HEPES (11,9g/l)	
P - Medium	0.20 %	peptone (Marcor M)	pH 7,5
	0.80 %	starch (Cerestar)	
	0.40 %	Probion	
	0.20 %	yeast extract (Marcor type 9000)	
	0.10 %	$CaCl_2$.$2H_2O$	
	0.10 %	MgSO4 · 7H2O	
	50 mM	HEPES (11,9g/l)	
	8 mg/L	Fe-EDTA	
S-Medium	0.40 %	soy flour (degreased)	pH 7,4
	0.20 %	glucose monohydrate	
	0.80 %	starch (Cerestar)	
	0.10 %	$CaCl_2$.$2H_2O$	
	0.10 %	MgSO4 · 7H2O	
	50 mM	HEPES (11,9g/l)	
	8 mg/L	Fe-EDTA	
Mxx-Medium	1 %	Casein peptone	pH 7,0
	0.01 %	CaCl2 x 2H2O	
	0.03 %	MgSO4 x 7H2O	
	1 mg/L	CoCl2	
	100 mM	HEPES (11,9g/l)	
Modified MA Medium	5 %	peptone (Marcor M)	pH 7,0
	1 %	yeast extract (Difco)	
	9 %	MgSO4 · 7H2O	
	4 %	NaSO4	
	2 %	CaCl2	
SAP2 Medium	1 %	Tryptone	
	1 %	yeast extract (Difco)	

VY/2 Modified Medium	5 %	Baker's yeast*	
RL1 Medium	3 %	peptone (Marcor M)	
	2 %	yeast extract (Difco)	
	0.5 %	KNO3	
	0.5 mg/L	vitamin B12	is sterilized by filtration
SK Medium	5 g	Skim milk	
	3 g	yeast extract (Difco)	
	1 g	MgSO4	

Table A4. (Minimum Inhibited Concentration) MIC Table

No.	Pathogenic microorganism	Medium	Inoculated in 20 ml med (µl)	Incubation (°C)	Antibiotic References
1	*Candida albicans* DSM 1665	Myc_1	90	30	Nystatin
2	*Acinetobacter baumannii* DSM 30008	Müller/Hinton	62	30	-
3	*Escherechia coli* WT BW25113	Müller/Hinton	59	37	Gentamycin
4	*Escherechia coli* acrB JW0451-2	Müller/Hinton	77	37	Gentamycin
5	*Citrobacter freundii* DSM30039	Müller/Hinton	63	30	-
6	*Mycobacterium smegmatis* ATCC70008 4	Middlebrook	150	37	Kanamycin
7	*Pseudomonas aeruginosa* PA14	Müller/Hinton	63	30 or 37	Gentamycin
8	*Staphylococcus aureus* Newmann	Müller/Hinton	43	30	Gentamycin
9	*Mucor himalis* DSM2656	Myc_1	150	30	Nystatin
10	*Wickerhamomyces anomalus* DSM6766	Myc_1	38	30	Nystatin

Table A5. HPLC parameter for fractionation protocol's

Method	**Fraktonierung_20 µL.M**
Temperature of Column	40 °C
Flow rate of solvent	0.3 mL / min
Mobile phase A	385 mg ammonium acetate + 40 µL acetic acid in 1 L water
Mobile phase B	acetonitrile with water (95: 5)

Table A6. API ZYM and API CAMPY

Well Number for APIZYM	Substrat	Enzyme
1	Negativkontrolle	-
2	2-Naphtylphosphat	Alkalische Phosphatasen
3	2-Naphtylburat	Esterase (C4)
4	2-Naphthylcaprylat	Esterase-Lipase (C8)
5	2-Naphthylmyristat	Lipase (C14)
6	L-Leucyl-2-Naphthylamid	Leucin-Arylamidase
7	L-Valyl-2-Naphthylamid	Valin-Arylamidase
8	L-Cystyl-2-Naphthylamid	Cytin-Arylamidase
9	N-Benzoyl-DL-Arginin-2-Naphthylamid	Trypsin
10	N-Glutarylphenylalanin-2-Naphthylamid	Chymotrypsin
11	2-Naphthylphosphat	Saure Phosphatase
12	Naphthol-AS-BI-Phosphat	Naphtol-AS-BI-Phosphohydrolase
13	6-Br-2-Naphthyl-D-Galactopyranosid	α-Galactosidase
14	2-Naphthyl-D-Galactopyranosid	β-Galactosidase
15	Naphthol-AS-BI-D-Glucuronid	β-Glucuronidase
16	2-Naphthyl-D-Glucopyranosid	α-Glucosidase
17	6-Br-2-Naphthyl-DGlucopyranosid	β-Glucosidase
18	1-Naphthyl-N-acetyl-D-Glucoseaminid	N-Acetylglucoseamidase
19	6-Br-2-Naphthyl-D-Mannopyranosid	α-Mannosidase
20	2-Naphthyl-L-Fucopyranosid	α-Fucosidase

Well Number for APICAMPY	Substrat	Reaction	Results	
			Negative	Positive
1	Urea	Urease	Yellow	Orange-Red
2	Potassium nitrate	Nitrate Reduction	Colorless	Pink-Red
3	5-Bromo-4-chloro-3-indoxyl- acetate	Esterase	Colorless, Black Blue	Turquoise
4	Sodium hippurate	Hipurate	Colorless, bluish-Gray	Violet
5	γL-glutamic acid- β-naphthylamide	Gamma-Glutamyltransferase	Colorless	Strong Oranges
6	Triphenyltetrazoliumchloride	Reduktion von Triphenyltetrazolium Chloride	Colorless, weak pink	Pink-Red
7	Pyroglutamic acid-β-Naphthylamide	Pyrrolidonyl Arylamidase	Colorless	Orange
8	L-Arginine-4-methoxy-β-Naphthylamide	L-Arginine Arylamidase	Colorless	Orange
9	Aspartic acid-β-Naphthylamide	L-Aspartate Arylamidase	Colorless	Orange
10	2-Naphtyl Phosphatase	Alkaline Phosphatase	Colorless	Violet
11	Hydrogen sulfide	Production from Hydrogen sulfide	Colorless	Black

Table A7. Gen III Microplate Biolog System

A1 Negative Control	A2 Dextrin	A3 D-Maltose	A4 D-Trehalose	A5 D-Cellobiose	A6 Gentiobiose	A7 Sucrose	A8 D-Turanose	A9 Stachyose	A10 Positive Control	A11 pH 6	A12 pH 5
B1 D-Raffinose	B2 α-D-Lactose	B3 D-Melibiose	B4 β-Methyl-D-Glucoside	B5 D-Salicin	B6 N-Acetyl-D-Glucosamine	B7 N-Acetyl-β-D-Mannosamine	B8 N-Acetyl-D-Galactosamine	B9 N-Acetyl Neuraminic Acid	B10 1% NaCl	B11 4% NaCl	B12 8% NaCl
C1 α-D-Glucose	C2 D-Mannose	C3 D-Fructose	C4 D-Galactose	C5 3-Methyl Glucose	C6 D-Fucose	C7 L-Fucose	C8 L-Rhamnose	C9 Inosine	C10 1% Sodium Lactate	C11 Fusidic Acid	C12 D-Serine
D1 D-Sorbitol	D2 D-Mannitol	D3 D-Arabitol	D4 myo-Inositol	D5 Glycerol	D6 D-Glucose-6-PO4	D7 D-Fructose-6-PO4	D8 D-Aspartic Acid	D9 D-Serine	D10 Troleandomycin	D11 Rifamycin SV	D12 Minocycline
E1 Gelatin	E2 Glycyl-L-Proline	E3 L-Alanine	E4 L-Arginine	E5 L-Aspartic Acid	E6 L-Glutamic Acid	E7 L-Histidine	E8 L-Pyroglutamic Acid	E9 L-Serine	E10 Lincomycin	E11 Guanidine HCl	E12 Niaproof 4
F1 Pectin	F2 D-Galacturonic Acid	F3 L-Galactonic Acid Lactone	F4 D-Gluconic Acid	F5 D-Glucuronic Acid	F6 Glucuronamide	F7 Mucic Acid	F8 Quinic Acid	F9 D-Saccharic Acid	F10 Vancomycin	F11 Tetrazolium Violet	F12 Tetrazolium Blue
G1 p-Hydroxy-Phenylacetic Acid	G2 Methyl Pyruvate	G3 D-Lactic Acid Methyl Ester	G4 L-Lactic Acid	G5 Citric Acid	G6 α-Keto-Glutaric Acid	G7 D-Malic Acid	G8 L-Malic Acid	G9 Bromo-Succinic Acid	G10 Nalidixic Acid	G11 Lithium Chloride	G12 Potassium Tellurite
H1 Tween 40	H2 γ-Amino-Butryric Acid	H3 α-Hydroxy-Butyric Acid	H4 β-Hydroxy-D,L-Butyric Acid	H5 α-Keto-Butyric Acid	H6 Acetoacetic Acid	H7 Propionic Acid	H8 Acetic Acid	H9 Formic Acid	H10 Aztreonam	H11 Sodium Butyrate	H12 Sodium Bromate

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